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Certification of Trace Elements and Methylmercury Mass Fractions in Fish Homogenate IAEA-476

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CERTIFICATION OF TRACE ELEMENTS AND METHYLMERCURY MASS FRACTIONS IN FISH HOMOGENATE IAEA-476

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CERTIFICATION OF TRACE ELEMENTS AND METHYLMERCURY MASS FRACTIONS IN FISH HOMOGENATE IAEA-476

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FOREWORD

Many IAEA Member State laboratories produce the local or regional monitoring results used for assessment of the marine environment or seafood safety. These laboratories are developing new analytical procedures for investigating the environmental impact of human activities, and for providing analytical services to other organizations. Because of the need to base scientific conclusions on valid and internationally comparable measurement results, and the need to provide policy makers with correct information on the state of the environment, it is critical that the quality of the measurement results produced by each laboratory be ensured. Since the 1960s, the IAEA has been assisting Member States in the field of data quality and quality assurance. To support Member States in both their marine monitoring activities and in the domain of food safety, the Marine Environmental Studies Laboratory produces certified reference materials characterized for trace elements and methylmercury using samples of marine origin biota and sediments. Certified reference materials are valuable tools for developing and validating analytical methods to improve the quality of measurement results.

This publication describes the methodologies for sample preparation and the assignment of property values for a number of trace elements in a marine biota sample. The new certified reference material, IAEA-476, was produced following the international guidelines on the production of certified reference materials. Thirteen laboratories with demonstrated experience in the field participated in the characterization of this fish homogenate sample.

The IAEA is grateful to the Government of Monaco for the support provided to the Environment Laboratories, and to the participants and laboratories that took part in this characterization study and contributed to the production process of the IAEA-476. The Marine Environmental Studies Laboratory in Monaco is grateful to the Radiometrics Laboratory for providing the raw material used. The IAEA officers responsible for this publication were E. Vasileva and S. Azemard of the IAEA Environment Laboratories.

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CONTENTS

1. INTRODUCTION

The use of suitable CRM is mandatory for the quality assurance of any measurement result produced by a laboratory, as well as its traceability to the common system of references –SI system. This is the reason why it is recommended to select a CRM that matches the analyzed sample with respect to the matrix and concentration level of elements of interest.

Fish filets are often analyzed in monitoring studies as well as for food safety purposes, therefore it is important to produce CRMs with marine fish origin. CRM IAEA-476 will assist laboratories in validating their analytical methods and controlling the quality of produced analytical results for the determination of trace elements and methyl mercury in marine fish samples [1].

The work presented in this report refers exclusively to the certification of the mass fractions of trace elements and methylmercury in the fish flesh homogenate matrix.

2. METHODOLOGY

2.1. PREPARATION OF THE MATERIAL

About 350 kg sample of mixed fish species was collected in the eastern Irish Sea by the CEFAS, Lowestoft, U.K. The fishes were deskinned, filleted, freeze-dried and subsequently sent to IAEA-NAEL for processing. The sample, which was further reduced by freeze-drying to about 69 kg, was ground to powder, sieved through a 0.5 mm mesh and homogenized by mixing in a nitrogen atmosphere. The sample was freeze-dried once again, ground and sieved at 250 µm and the fraction above 250 µm ground by micronisation. As can be seen in Figure 1 after reprocessing the size of 95% of sample particles was below 160 µm. The obtained powder was further homogenized for several days and bottled in sealed plastic containers.

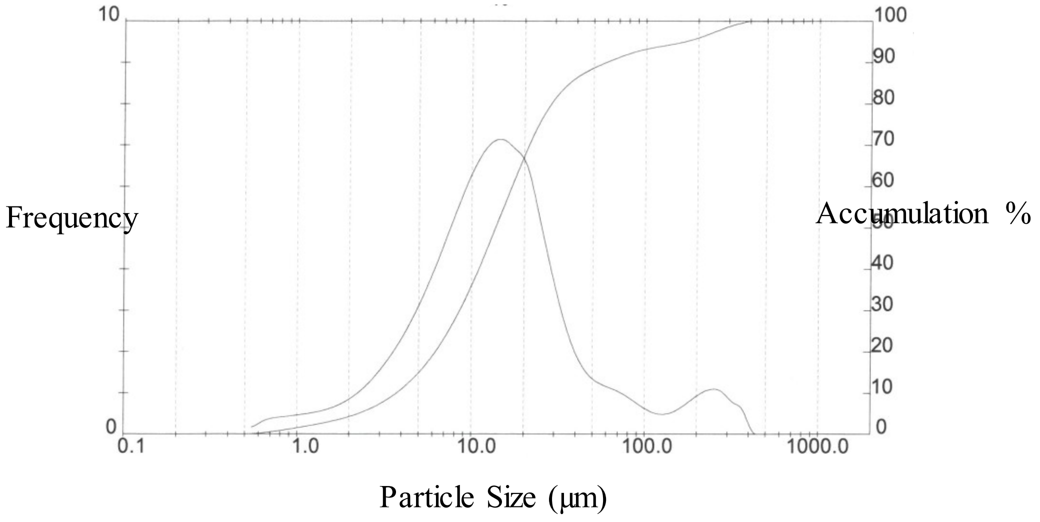

FIG. 1. Grain size distribution of final sample.

2.2. SELECTION OF LABORATORIES

The selection of participants for the characterization study was based on the measurement performances demonstrated by laboratories in the previous three ILCs, organized by the IAEA. Only results of laboratories having a quality system in place, using validated methods, applying uncertainty and traceability concepts were used for the calculation of the assigned values and their uncertainties [2]. Each participant or working group was identified with a code number.

The list of laboratories participating in the IAEA 476 characterization study is presented on page 39.

2.3. HOMOGENEITY ASSESSMENT

Extensive homogeneity tests were carried out on the fish homogenate in order to estimate the uncertainty contribution coming from the inhomogeneity of the sample and to ensure its suitability as a certified reference material.

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all produced units, within the stated uncertainty. The between-unit homogeneity was tested by the determination of the mass fractions of Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, CH_3Hg, Ni, Pb, Se, Sr, V and Zn in the sample.

In total, 14 bottles from the whole batch were selected, using random stratified sampling. Duplicate subsamples from each bottle were analyzed for their total element mass fractions. For all analytes except Hg and CH_3Hg, subsamples of 0.2 g were mineralized with 5 ml conc. HNO_3 in a microwave oven. The final measurements were performed by Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) or Flame Atomic Absorption Spectrometry (AAS) under repeatability conditions, and in a randomized way. The determination of the total Hg was done in solid subsamples (50 mg) with an Advanced Mercury Analyzer (AMA). Methyl mercury was determined by Gas Chromatography- Atomic Fluorescence Spectrometry (GC-AFS) after alkaline digestion and room temperature derivatization.

The results were corrected for the water content determined in each unit by using the procedure in the Section 2.6.

All methods used for homogeneity studies were previously validated in IAEA, MESL, inorganic chemistry laboratories.

2.4. STABILITY STUDY

At the time of bottling (May 2016) 5 bottles have been stored under so called “reference” condition: (-20 ± 2) °C in the dark. The other produced unit were then stored under “ambient" conditions: (+20 ± 3) °C in the dark.

To evaluate potential degradation of the material over 1 year, subsamples from 3 bottles stored under "reference" conditions were analyzed together with the samples from the homogeneity study. Obtained data for "reference" units was compared to results obtained for other units (analyzed for the dual purpose of homogeneity and stability study) to evaluate potential degradation of the material over one year. Duplicates subsamples from each bottle were analyzed for their total element mass fractions as describe in the Section 2.3.

The measurements were performed under repeatability conditions and in randomized way in order to separate a potential analytical drift from a trend related to the storage conditions. The results were corrected for the water content, determined in each unit, applying the protocol described in the Section 2.6.

2.5. CHARACTERIZATION

The fish sample was initially analyzed in the IAEA-EL in Monaco. The final characterization was based on the results delivered by selected laboratories with demonstrated measurement capabilities (2.2).

Each laboratory received one bottle of fish flesh homogenate sample, accompanied by an information sheet and a reporting form. Participants were requested to analyze Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, CH_3Hg, Ni, Pb, Se, Sr, V and Zn using validated analytical methods. They were asked to report measurement results (three replicates and average value), expanded uncertainty and the information on the applied quality control procedures. In addition, they were requested to provide results for the mass fractions of the analyzed trace elements in one CRM with matrix composition similar to the matrix of the candidate reference material, as well as the information on the standard calibration solutions used in the measurement step.

As the result for the moisture content in the fish sample is an operationally dependent parameter, the method for moisture determination was prescribed to all participating laboratories.

The results of laboratories not fulfilling the above described requirements were excluded from the further evaluation. Datasets with noncompliance to the previously defined criteria or considered as not technically valid are listed in Table 1.

The characterization of the trace elements mass fractions in the sample was based on the application of several analytical techniques. They are summarized in Figure 2. Abbreviations of the instrumental techniques applied in this characterization study are given in Table 2.

TABLE 1. Dataset excluded from further evaluation after technical review

Element	Lab code	Description
As	12 and 13	high uncertainty (>20%) when compared to other expert laboratories
Hg	12 and 13	high uncertainty (>20%) when compared to other expert laboratories
Se	13	high uncertainty (>20%) when compared to other expert laboratories
Zn	12	high uncertainty (>20%) when compared to other expert laboratories

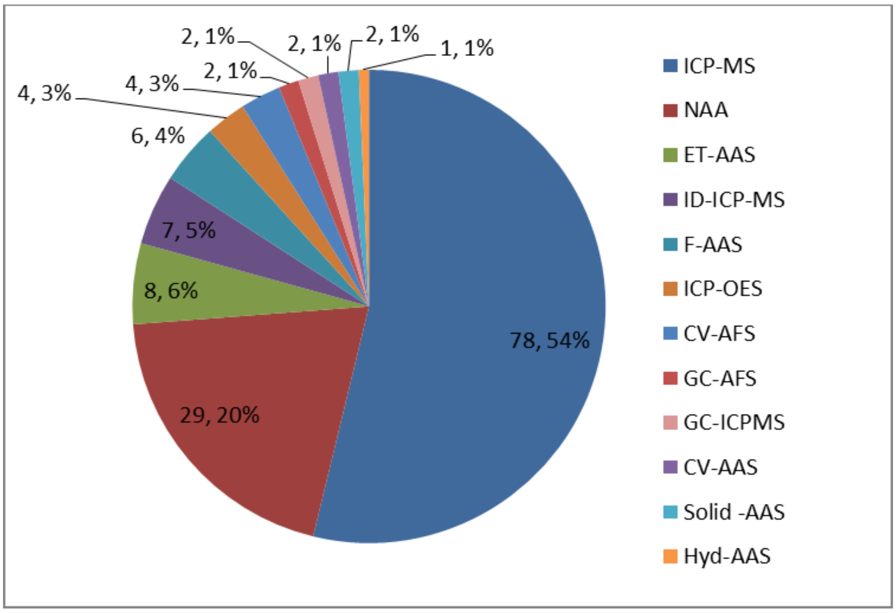

FIG. 2. Analytical methods used for the characterization of trace elements in the IAEA-476.

TABLE 2. ABBREVIATION FOR INSTRUMENTAL TECHNIQUES

Abbreviation	Instrumental technique
AAS	Atomic Absorption Spectrometry
AFS	Atomic Fluorescence Spectrometry
CV	Cold Vapor
Hyd	Hydride
GC	Gas Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
F	Flame
ET	Electro Thermal
NAA	Neutron Activation Analysis
ID-ICP-MS	Isotope Dilution Inductively Coupled Plasma Mass Spectrometry

2.6. MOISTURE DETERMINATION

The determination of the moisture content of the samples is to some extent an ‘operationally defined’ parameter. In view of the comparability of results, the protocol for the correction for moisture was developed at the IAEA and prescribed to other participants. The drying procedure at (85 ± 2) °C was established after experimental evaluation of sample stability.

Correction for dry mass was obtained by repeated drying of separate subsamples (0.5 g each) at 85°C until the constant mass is attained (usually 24 hours). Moisture, determined at MESL (10 subsamples from 5 bottles) was found to be (7.6 ± 0.5) % for bottles kept at 20°C.

3. RESULTS AND DISCUSSION

3.1. RESULTS OF HOMOGENEITY STUDY

3.1.1. Between-unit homogeneity

For the homogeneity study, 14 units of the sample were selected by using a random stratified sample selecting scheme and analyzed for their trace elements contents in duplicate.

Regression analyses were performed to evaluate potential trends in the analytical and processing sequences. Grubbs and Dixon tests at 95% and 99% confidence levels were performed to identify potentially outlying individual results or bottle means. As a prerequisite for the application of ANOVA for the estimation of uncertainty arising from homogeneity, it was verified whether the individual results and unit means follow a normal distribution or are unimodally distributed.

Individual outliers were detected for Ag, Cu, CH_3Hg and Zn. The outliers detected for Ag and Cu were linked to the contamination during analytical run and were rejected before the statistical treatment. In the case of Zn and CH_3Hg reported results were normally distributed. No technical reasons were found for the observed single outliers and all reported for Zn and CH_3Hg results were retained for further statistical treatment.

Significant analytical sequence trend was found for Cd, Co, Cr, Cu, Ni, V and Zn. A linear model was chosen as a reasonable approximation. Before other statistical treatment results were corrected following the Eq. 1.

$$Corrected\ result = Measured\, Result - (b \times i) \tag{1}$$

Were b is the slope of the linear model and i is the position of the analyzed subsample in the run.

Quantification of between-unit homogeneity was done by one-way analysis of variance (ANOVA) which can separate the between-unit variation from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability, if the individual aliquots are representative for the whole unit.

Using ANOVA the between unit standard deviation (s_{bb}) can be computed from the between-group mean square (MS_{bb}), the within-group mean square (MS_{wb}), and the number of replicate per unit (n) using the equation described below:

$$s_{wb} = \sqrt{MS_{wb}} \tag{2}$$

$$s_{bb} = \sqrt{\frac{MS_{bb} - MS_{wb}}{n}} \tag{3}$$

s_{bb} and s_{wb} are estimates of the standard deviations and are therefore subject to random fluctuations. Therefore, the mean square between groups can be smaller than the mean squares within groups, resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum heterogeneity, that could be hidden by method repeatability, was calculated as described by Linsinger et al. [4]. u^*_{bb} is comparable to the limit of detection of an analytical method, yielding the maximum heterogeneity that might be undetected by the applied experimental setup.

$$u^*_{bb} = \sqrt{\frac{MS_{wb}}{n}} \cdot \sqrt[4]{\frac{2}{\nu_{MS_{wb}}}} \tag{4}$$

Where: n is the number of replicate sub-samples per bottle; and $\nu_{MS_{wb}}$ is the degrees of freedom of MS_{wb}.

For Co, Cr, Cu, Fe and Ni a significant processing trend was observed, in that case the estimate of heterogeneity between bottles was modeled as the half with of a rectangular distribution following Eq.5.

$$u_{rect} = \frac{highest\ result - lowest\ results}{2\sqrt{3}} \tag{5}$$

For V one bottle mean was detected as outlier, between bottle heterogeneity was modeled as the rectangular distribution limited by the outlying average using Eq.6.

$$u_{rect} = \frac{|outlier - y|}{\sqrt{3}} \tag{6}$$

With y = average of all results.

For Ag, As, Cd, Hg, CH_3Hg, Pb, Se, Sr and Zn, ANOVA was applied and uncertainty contribution of the between-unit homogeneity (u_{hom}) was estimated according to the ISO Guide 35 [3] as the maximum values obtained with the Eq. 3 or Eq. 4. Obtained results are presented in Table 3. As can be seen in Table 3 the between-unit variations for Ag, As, Cd, Hg, Mn, CH_3Hg, Pb, Se and Zn were sufficiently small to demonstrate the homogeneity of the material for specified sample mass.

The between-unit variations for Co, Cr, Ni, Sr and V were in the range 8-14 % demonstrating unacceptable heterogeneity of the material for those elements.

TABLE 3. THE ESTIMATE OF HOMOGENEITY CONTRIBUTIONS TO THE TOTAL UNCERTAINTY FOR THE CERTIFIED TRACE ELEMENTS

	s_{wb}	s_{bb}	u^*_{bb}	u_{rect}	u_{hom}
Ag	4.6%	2.8%	2.0%		2.8%
As	5.6%	2.9%	2.5%		2.9%
Cd	6.4%	[1]	2.8%		2.8%
Co				12.3%	12.3%
Cr				11.2%	11.2%
Cu				5.80%	5.80%
Fe				6.40%	6.40%
Hg	1.19%	0.56%	0.52%		0.56%
CH_3Hg	3.4%	2.2%	1.5%		2.2%
Mn	1.8%	4.3%	0.8%		4.3%
Ni				14.0%	14.0%
Pb	5.6%	2.9%	2.5%		2.9%
Se	10.7%	[1]	4.6%		4.6%
Sr	3.4%	8.1%	1.5%		8.1%
V	7.6%	2.7%	3.4%	8.50%	8.5%
Zn	2.0%	2.2%	0.9%		2.2%

1) Not defined due to negative argument under the square root

3.1.2. Within-unit homogeneity

The within-unit homogeneity is closely related with the minimum sample intake. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus can be used in an analysis. Sample sizes equal or above the minimum sample intake guarantee the assigned value within its stated uncertainty. During characterization study the minimum sample size was prescribe to participant (0.05g for Hg and 0.2g for other trace element), based on preliminary homogeneity study.

The conclusion from the presented results was that the homogeneity of the fish sample complied with the provisions given by the ISO Guide 35 [3] at the range of weights used. A minimum sample intake of 0.2 g for Ag, As, Cd, Cu, Fe, Mn, CH_3Hg, Pb, Se, Sr, and Zn and 0.05g for Hg was set.

3.2. RESULTS FOR STABILITY STUDY

The samples selected for long term stability study were analyzed and each of the elements (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, CH_3Hg, Ni, Pb, Se, Sr, V and Zn) was evaluated individually.

The evaluation of data was further carried out by performing a t-test assuming equal variance. Except for Sr no statistical differences were detected between results obtained in units stored under "normal" or "reference" conditions.

Based on experience of long term monitoring study (more than 10 years) of previous IAEA reference material of same matrix, and as the original material was prepared more than 20 years ago, the material is expected to be stable and the uncertainty associated with stability (u_{stab}) was set to zero [3].

Graphical representations of the long-term stability study are displayed in Appendix I (Figures 3– 18).

3.3. DETERMINATION OF ASSIGNED VALUES AND THEIR UNCERTAINTIES

The characterization campaign resulted in 2 to 12 measurement results for the requested trace elements. The obtained measurement results were first checked for compliance with the certification requirements, and then for their validity based on technical reasoning. All accepted sets of results were submitted to the following statistical tests: Grubbs and Dixon's test to detect outliers and Kolmogorov-SmirNov's test for Normal distribution. As shown in Table 4, all data sets were normally distributed and outliers were found for Cd, Co, Zn. No technical reasons were identified for outlying results, all data were retained for statistical analysis.

The medians, unweighted mean of the means and robust mean were calculated and compared (Table 4). No significant differences were observed and the reference values obtained with the mean of the mean approach was further used. These values are considered to be the most reliable estimates of the property values of the selected trace elements in fish homogenate.

TABLE 4. COMPARISON OF DIFFERENT MEAN

Element	Robust Mean** (mg kg^{-1})	Median (mg kg^{-1})	Mean of the means (mg kg^{-1})	Outlier 95%	Outlier 99%	Distribution
Ag	0.058	0.058	0.058	0	0	Normal
As	21.2	21.4	21.2	0	0	Normal
Cd	0.029	0.029	0.028	1	0	Normal
Co	0.112	0.098	0.134	1	1	Normal
Cr	3.67	3.30	3.77	0	0	Normal
Cu	2.39	2.35	2.38	0	0	Normal
Fe	140	143	139	0	0	Normal
Hg	0.577	0.577	0.578	0	0	Normal
CH_3Hg*	0.523	0.530	0.523	0	0	Normal
Mn	16.5	16.4	16.5	0	0	Normal
Ni	4.23	4.19	4.23	0	0	Normal
Pb	0.653	0.659	0.644	0	0	Normal
Se	2.12	2.13	2.12	0	0	Normal
Sr	145	146	143	0	0	Normal
V	0.343	0.344	0.343	0	0	Normal
Zn	52.8	52.5	53.6	1	1	Normal

* mg kg^{-1} as Hg

** robust means were calculated as described in the ISO guide 13528 [5].

The uncertainties associated with the assigned values were calculated according to ISO Guide 35 [3]. The relative combined uncertainty of the certified value of the CRM consists of uncertainty related to characterization u_{char}, between-unit heterogeneity (u_{hom}) and long term stability (u_{stab}). These different contributions were combined to estimate the expanded uncertainty.

$$U = k \times \sqrt{u_{char}^2 + u_{stab}^2 + u_{hom}^2} \qquad (7)$$

Where

k: coverage factor equaling 2, representing a level of confidence of about 95%

u_{hom} was estimated as described in section 3.1.1.

u_{stab} was set at zero as already described in section 3.2

u_{char} was estimated as described in ISO 35 [3] using Eq. (8):

$$u_{char} = \frac{s}{\sqrt{p}} \tag{8}$$

Where: s is the standard deviation of the mean; p is the number of laboratories.

Means values, their expanded uncertainties (k=2) and uncertainty contributions from the characterization, homogeneity and stability studies are presented in Table 5 for all trace elements.

TABLE 5. MEAN OF THE MEAN AND UNCERTAINTIES

Element	Mean of the mean mg kg^{-1}	$u_{char,rel}$ %	$u_{hom,rel}$ %	$U_{,rel}$ (k=2) %
Ag	0.058	4.1	2.8	9.9
As	21.2	1.4	2.9	6.0
Cd	0.028	3.9	2.8	10.0
Cu	2.38	3.2	5.80	13.0
Fe	139	2.7	6.40	14.0
Hg	0.578	1.8	0.56	4.0
CH_3Hg*	0.523	1.9	2.2	6.0
Mn	16.4	2.4	4.3	10.0
Pb	0.644	3.0	2.9	8.0
Se	2.12	1.4	4.6	10.0
Zn	53.6	1.7	2.2	5.0

* mg kg^{-1} as Hg

The results for the mass fractions of the trace elements as reported by the participants in this characterization and grouped by methods are presented in Appendix II. In all figures the reported results are plotted versus the mean of the mean value denoted by a bold line, while the dashed lines represent the expanded uncertainty (k=2) associated (as calculated in Eq. 8). The error bars represent the expanded uncertainty as reported by participants.

As shown previously in Figure 2 and in in Figure 19-34, methods with different quantification steps (Graphite Furnace-AAS, AFS, ICP-MS) as well as methods without sample preparation step such as neutron activation or solid sampling AAS were used for the characterization of the material. A good agreement within the stated uncertainty was observed for results obtained with different method, therefore all of them were considered in deriving assigned values. The agreement between results confirms the absence of any significant method bias and demonstrates the identity of the analyte.

Certified values were calculated as the mean of the mean of accepted dataset for elements fulfilling the following criteria: at least 5 results from 2 different methods were available and relative expanded uncertainties of the assigned value less than 15% (k=2).

These criteria were fulfilled for Ag, As, Cd, Cu, Fe, Hg, CH_3Hg, Mn, Pb, Se and Zn. The certified values are presented in Table 6, together with their expanded uncertainty.

The above conditions were not satisfied for Co, Cr, Ni, Sr and V only information values were provided. They are presented in Table 7.

TABLE 6. CERTIFIED VALUES FOR TRACE ELEMENT MASS FRACTIONS AND THEIR EXPANDED UNCERTAINTY (*k*=2) IN IAEA-476

Element	Unit	Assigned value[1]	Expanded uncertainty (*k*=2)[2]
Ag	mg kg^{-1}	57.6 × 10^{-3}	5.7 × 10^{-3}
As	mg kg^{-1}	21.2	1.4
Cd	mg kg^{-1}	28.0 × 10^{-3}	2.7 × 10^{-3}
Cu	mg kg^{-1}	2.38	0.31
Fe	mg kg^{-1}	139	19
Hg	mg kg^{-1}	0.578	0.022
CH_3Hg	mg kg^{-1} as Hg	0.523	0.030
Mn	mg kg^{-1}	16.4	1.6
Pb	mg kg^{-1}	0.644	0.053
Se	mg kg^{-1}	2.12	0.20
Zn	mg kg^{-1}	53.6	2.9

[1] The value is the mean of the mean of the ccepted sets of data, each set being obtained by different laboratory. The certified values are reported on dry mass basis and are traceable to the SI.

[2] Expanded uncertainty with a coverage factor *k* =2 estimated in accordance with the JCGM 100:2008 Evaluation of measurement data – Guide to the expression of uncertainty in measurement [6], corresponding to the level of confidence of about 95%.

TABLE 7. INFORMATION VALUES FOR TRACE ELEMENTS MASS FRACTIONS AND THEIR EXPANDED UNCERTAINTY ($k=2$) IN IAEA-476

Element	Unit	Information value[1]	Expanded uncertainty (k=2)[2]
Co	mg kg^{-1}	0.134	0.065
Cr	mg kg^{-1}	3.8	1.1
Ni	mg kg^{-1}	4.2	1.3
Sr	mg kg^{-1}	143	24
V	mg kg^{-1}	0.34	0.11

[1] The value is the mean of the mean of accepted sets of data.

[2] Expanded uncertainty with a coverage factor $k=2$ estimated in accordance with the JCGM 100:2008 Evaluation of measurement data – Guide to the expression of uncertainty in measurement [6], corresponding to the level of confidence of about 95%.

4. METROLOGICAL TRACEABILITY AND COMMUTABILITY

Pure metal standard solutions (CRMs) with stated purity were employed for calibration by all laboratories participating in this characterization study. As stated in the respective certificates of all CRM producers, the mass fractions of the trace element in the respective standard solutions were measured against another CRM (i.e. NIST, BAM or EMPA) with demonstrated SI traceability, followed by gravimetric preparation using balances calibrated with SI-traceable weights.

Only validated methods applied within stated scope were used by participating laboratories in this characterization study. Matrix CRMs with stated SI traceability purchased from NIST, EC JRC IRMM, NRC-CNRC and IAEA were used for validation of the methods applied in this study.

In addition, the agreement between the results confirms the absence of any significant method bias and demonstrates the identity of the measurand. The participants used different methods for the sample preparation as well as for the final determination, demonstrating absence of measurement bias.

As the certified values are combinations of agreeing results, individually traceable to the SI, the certified quantity values are also traceable to the SI system of units. Therefore, individual assigned results are traceable to the SI. The trust in the certified values and their trueness are further underpinned by the agreement among the technically accepted datasets.

The degree of equivalence in the analytical behavior of real samples and a CRM with respect to various measurement procedures (methods) is summarized in a concept called 'commutability of a reference material'.

Commutability is a property of a reference material, demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two given measurement procedures, and the relation obtained among the measurement results for other specified materials [7].

The appropriate characterization of CRMs, especially those materials intended to be used with routine measurement procedures, must carefully address fitness-for-use for all methods which the material is intended to be used for. Commutability is a critical requirement to avoid introducing unintended, and sometimes undetected, bias results when using a CRM.

Commutable CRMs should exhibit an analytical behavior for a given method similar to a real laboratory sample. However, CRMs might show behavior different from that of real samples, in particular during digestion, due to their small particle size in contrast to the possible larger particle size for real laboratory samples. IAEA-476 is a natural marine biota sample. The analytical behavior should be the same as for a routine sample of dried biota samples. The agreement between results obtained with different analytical methods selected for the IAEA-476 characterization study confirms the absence of any significant method bias and demonstrates commutability of the material for all certified trace elements.

5. CONCLUSIONS

This certification campaign allows assignment of certified values for Ag, As, Cd, Cu, Fe, Hg, CH_3Hg, Mn, Pb, Se and Zn with associated uncertainties following ISO guidelines. The certified values are derived from measurement results provided by the laboratories participating in the characterization study. Only validated methods were applied in the characterization of IAEA-476 CRM. As the certified values are combinations of SI traceable individual results, they are also traceable to the International System of Units.

APPENDIX I

RESULTS FROM THE LONG TERM STABILITY STUDY:

Figures 3-18 present individual mass fractions measured in unit kept 1 year at reference temperature (-20°C); at normal temperature (+20°C).

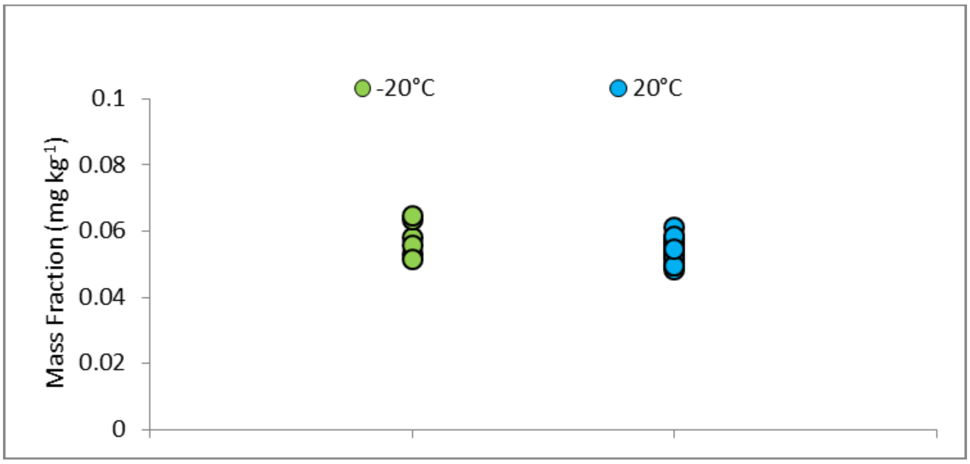

FIG. 3. Results of long term stability study for silver.

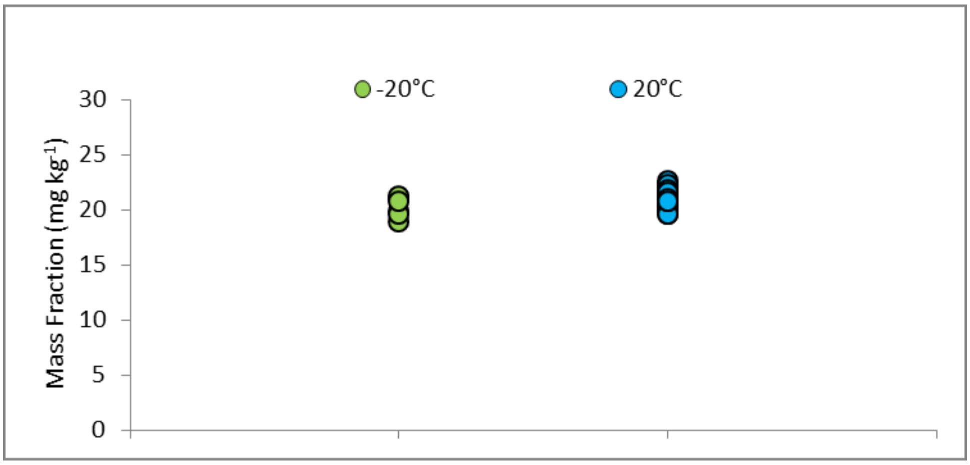

FIG. 4. Results of long term stability study for arsenic.

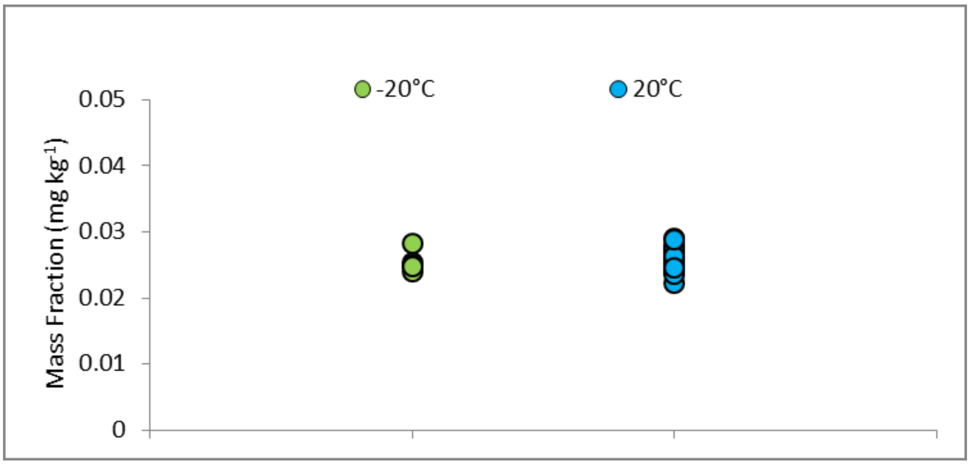

FIG. 5. Results of long term stability study for cadmium.

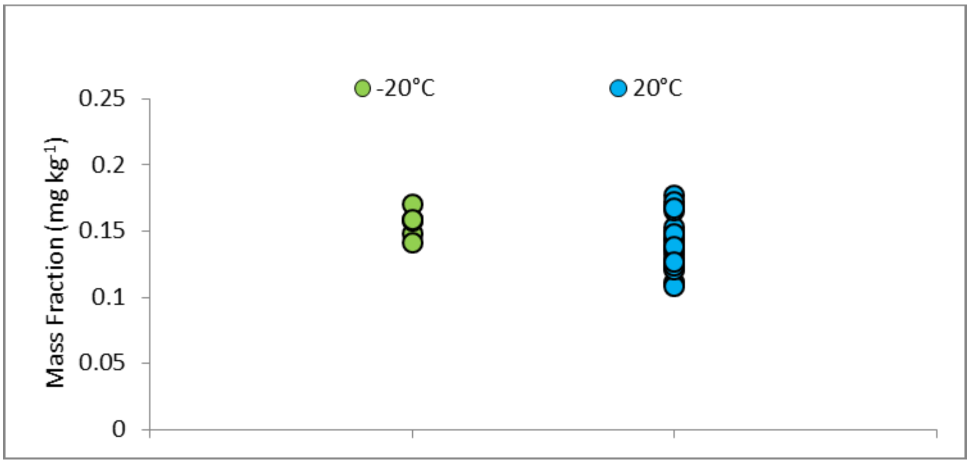

FIG. 6. Results of long term stability study for cobalt

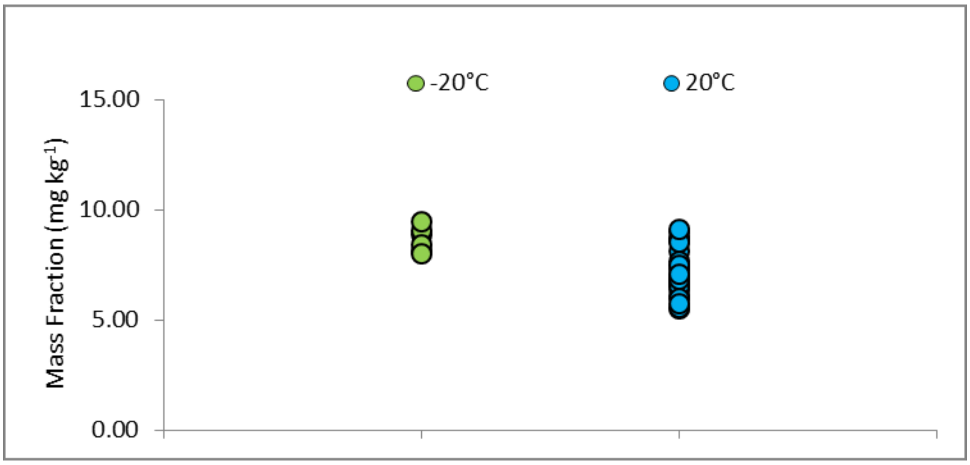

FIG. 7. Results of long term stability study for chromium.

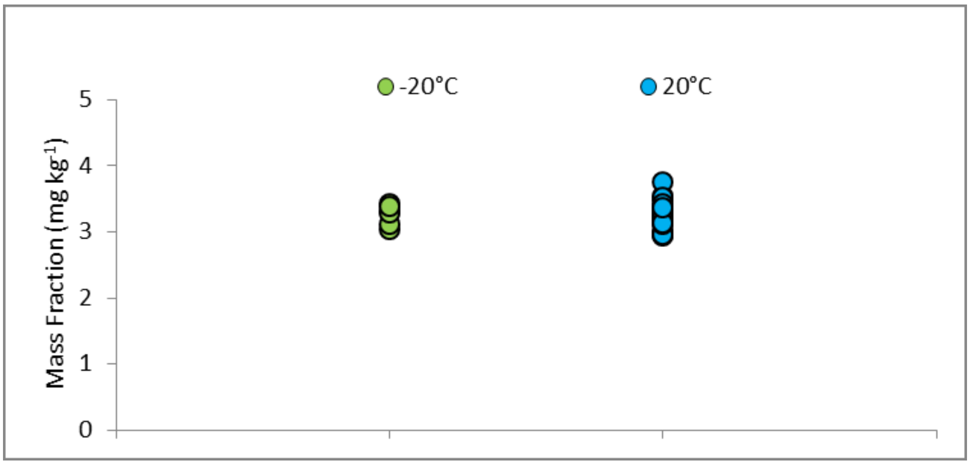

FIG. 8. Results of long term stability study for copper.

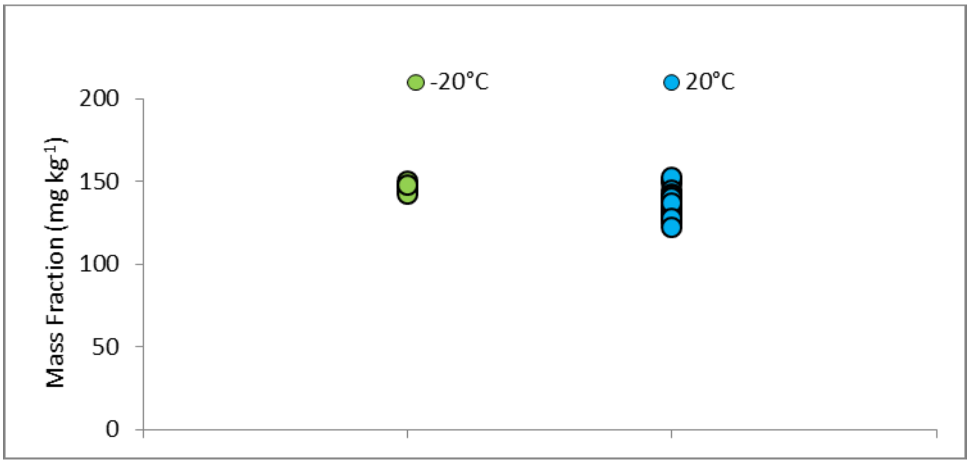

FIG. 9. Results of long term stability study for iron.

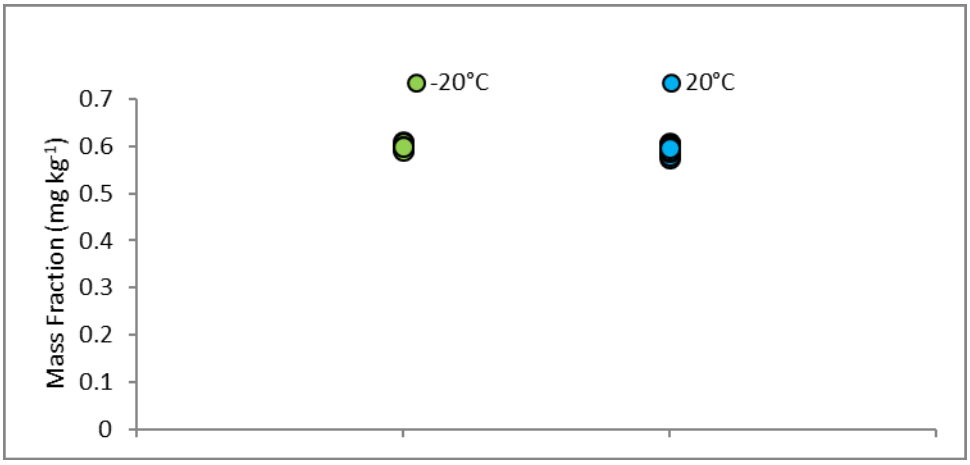

FIG. 10. Results of long term stability study for total mercury.

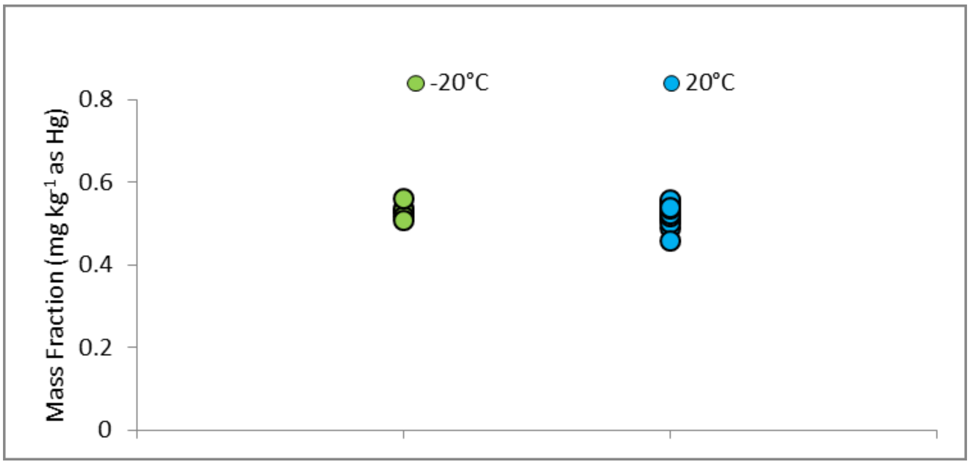

FIG. 11. Results of long term stability study for methyl mercury.

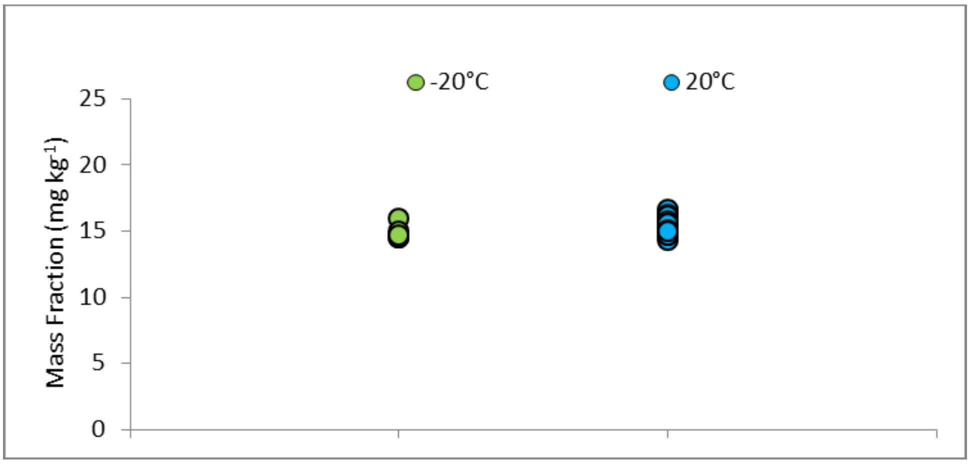

FIG. 12. Results of long term stability study for manganese.

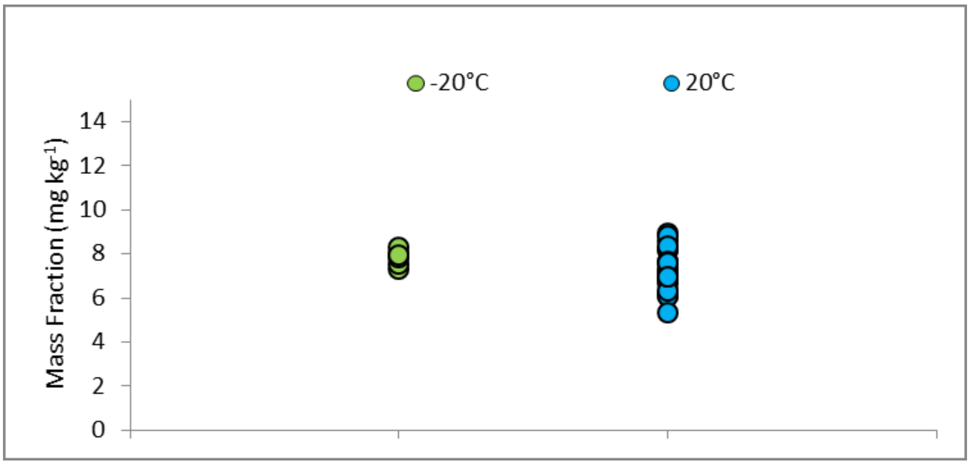

FIG. 13. Results of long term stability study for nickel.

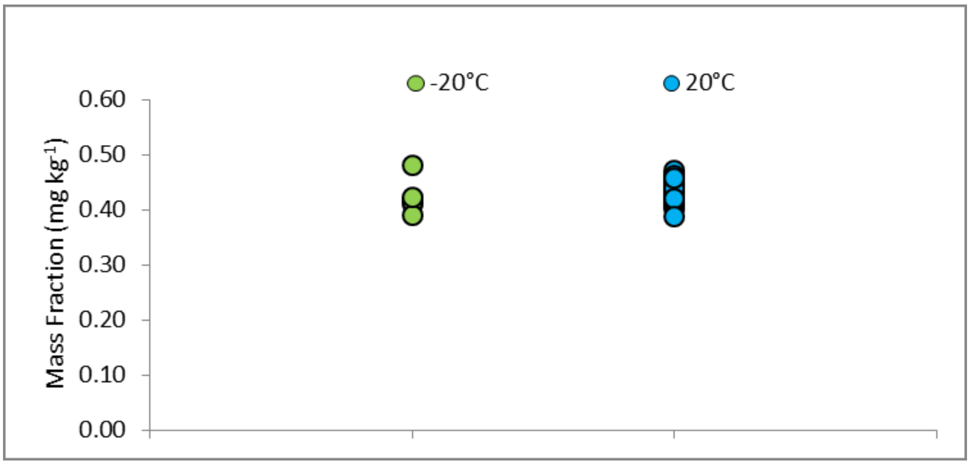

FIG. 14. Results of long term stability study for lead.

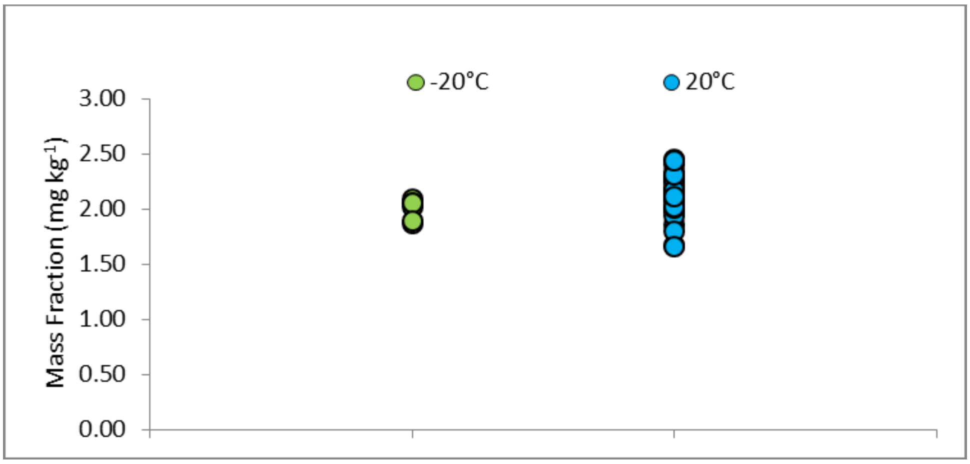

FIG. 15. Results of long term stability study for selenium.

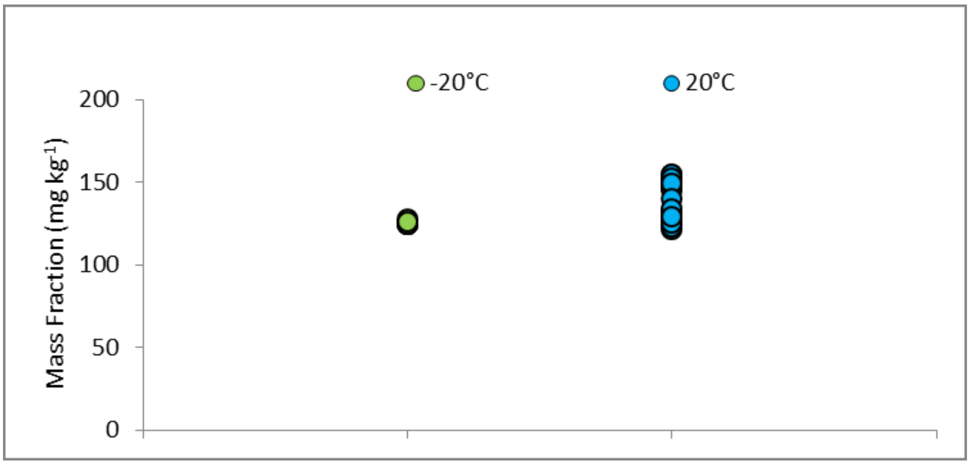

FIG. 16. Results of long term stability study for strontium.

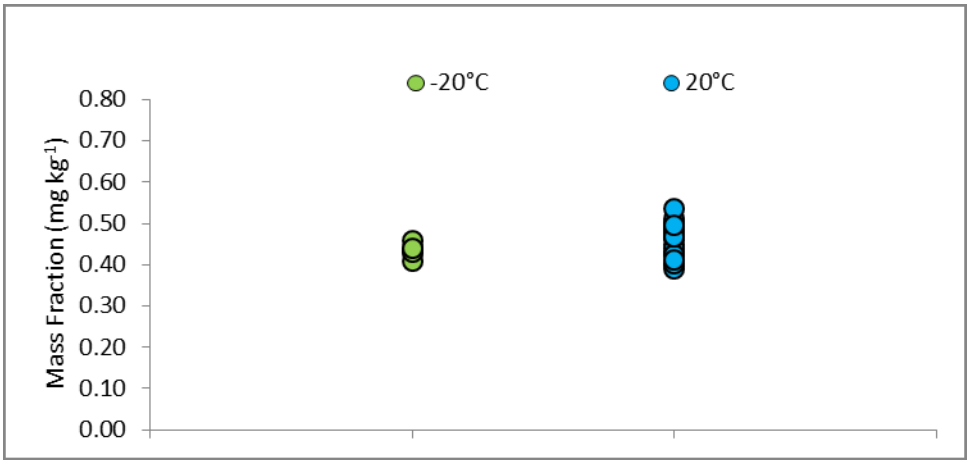

FIG. 17. Results of long term stability study for vanadium.

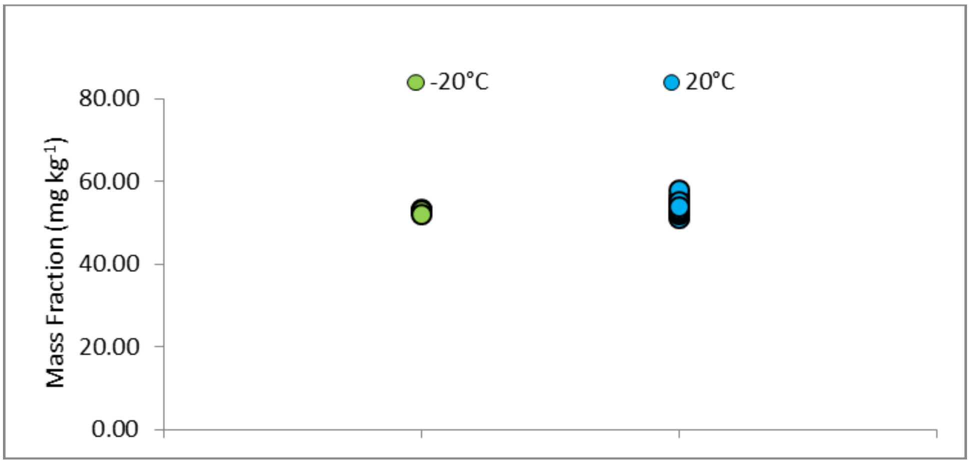

FIG. 18. Results of long term stability study for zinc.

APPENDIX II

RESULTS OF THE CHARACTERIZATION MEASUREMENTS

The reported by the participating results, their expended uncertainty, measurement techniques and CRMs used for quality assurance purposes are presented in Tables 8-23. Figures 19–34 provide graphical presentation of the individual results and their expanded uncertainties as well as the assigned value for the respective trace element and its expanded uncertainty (k=2), as a solid and dashed lines respectively.

TABLE 8. SILVER: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
6	0.055	0.027	DORM-2	ICP-MS
7	0.060	0.004	SRM 1566b	NAA
9	0.065	0.001	Blank Spike	ICP-MS
12	0.051	0.016	NIST1566b	ICP-MS
IAEA	0.058	0.007	IAEA 407	ICP-MS

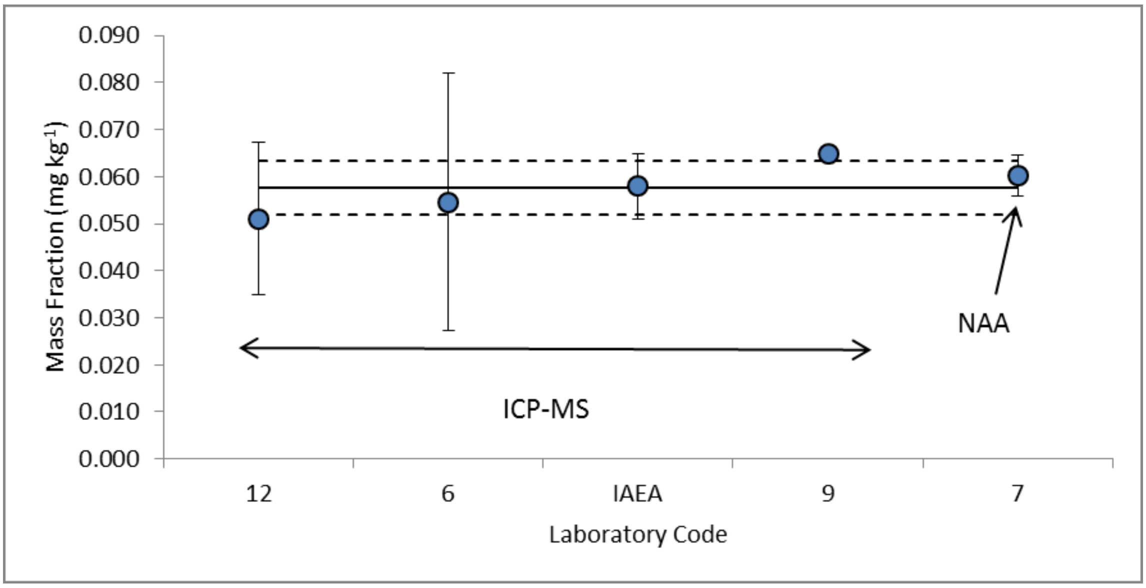

FIG. 19. Laboratory results for silver mass fraction (mg kg^{-1}) in IAEA-476

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TABLE 9. ARSENIC: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
3	22.2	1.6	NIST SRM-1547	NAA
5	21.2	1.7	IAEA 407	ET-AAS
6	21.7	3.3	DORM-2	ICP-MS
7	20.8	1.0	SRM 1566b	NAA
8	20.8	1.2	IAEA407	ICP-MS
9	20.7	1.8	TORT-3	ICP-MS
11	19.9	1.0	NIST 1566b	NAA
IAEA	21.4	3.0	IAEA 407	ICP-MS

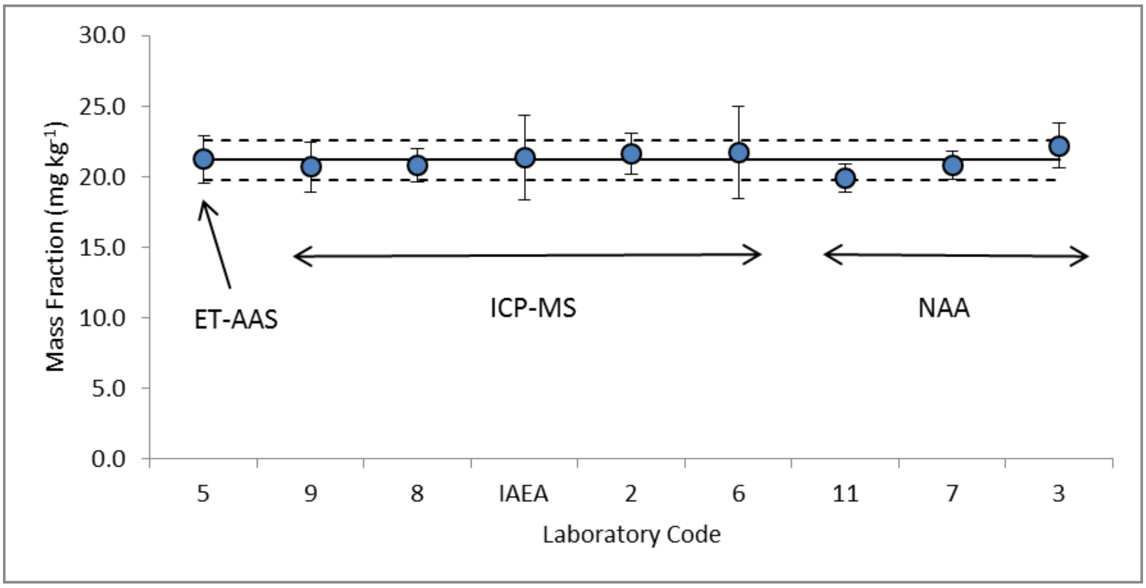

FIG. 20. Laboratory results for arsenic mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 10. CADMIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	0.021	0.009	DORM 3	ICP-MS
5	0.027	0.002	IAEA 407	ET-AAS
6	0.030	0.009	DORM-2	ICP-MS
8	0.030	0.003	IAEA436	ICP-MS
9	0.029	0.001	TORT-3	ICP-MS
12	0.025	0.005	NIST1566b	ICP-MS
IAEA	0.030	0.004	IAEA 407	ET-AAS
IAEA	0.032	0.004	IAEA 407	ICP-MS
IAEA	0.028	0.001		ID-ICP-MS

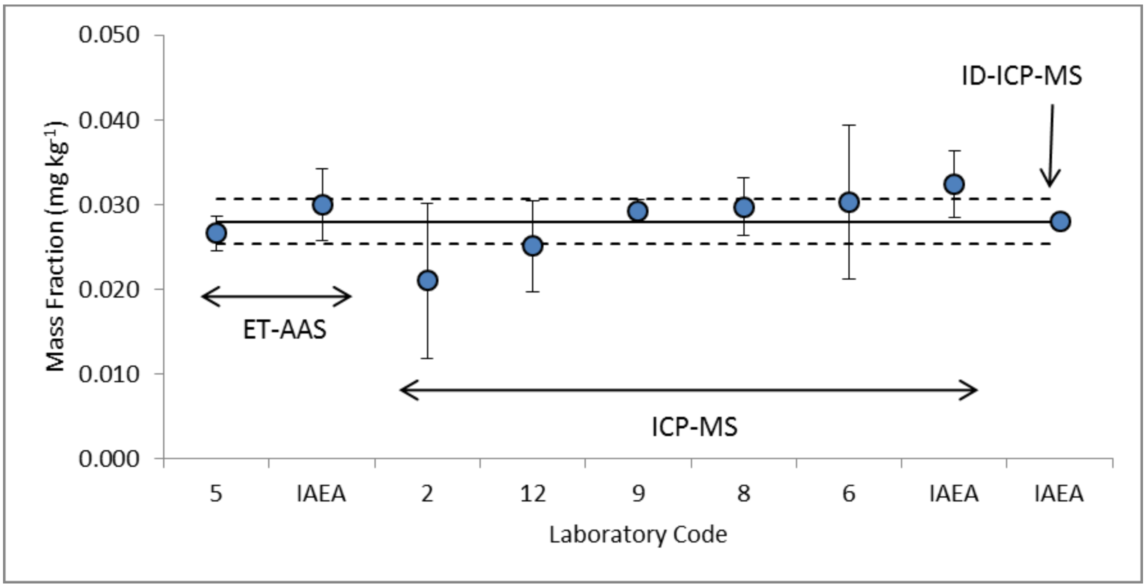

FIG. 21. Laboratory results for cadmium mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 11. COBALT: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
3	0.098	0.008	NIST SRM-1547	NAA
6	0.089	0.027	DORM-2	ICP-MS
7	0.101	0.006	SRM 1566b	NAA
8	0.133	0.017	IAEA407	ICP-MS
9	0.096	0.008	TORT-3	ICP-MS
11	0.295	0.005	NIST 1566b	NAA
12	0.076	0.018	NIST1566b	ICP-MS
IAEA	0.147	0.024	IAEA 407	ICP-MS

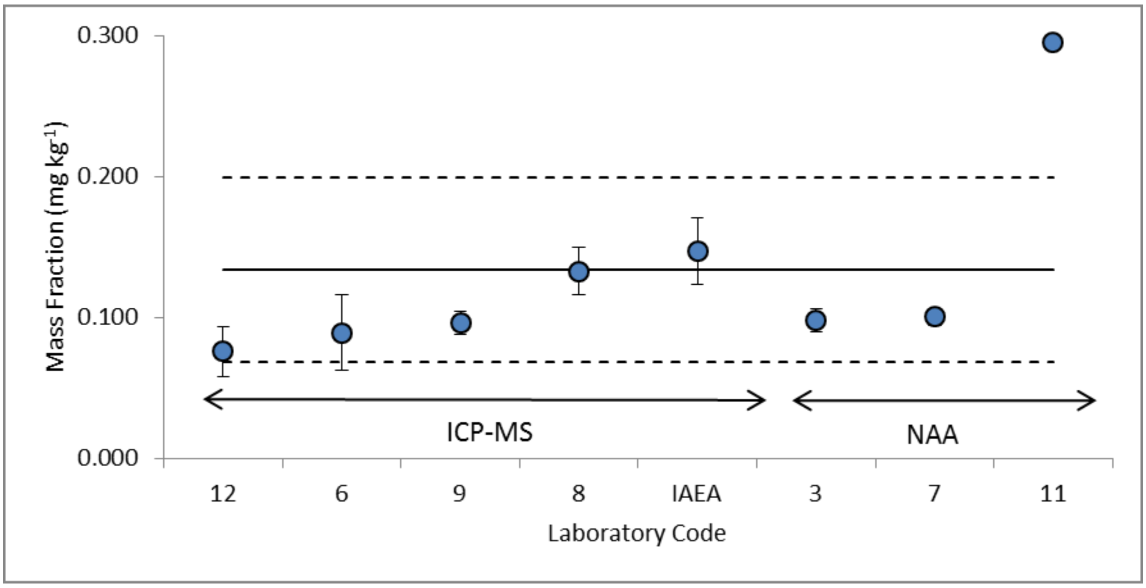

FIG. 22. Laboratory results for cobalt mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 12. CHROMIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	3.16	0.14	DORM 3	ICP-MS
3	4.96	0.40	NIST SRM-1547	NAA
6	3.96	0.59	DORM-2	ICP-MS
7	5.01	0.26	SRM 1566b	NAA
9	5.05	0.57	TORT-3	ICP-MS
11	3.30	0.20	NIST 2976	NAA
12	3.26	0.71	NIST1566b	ICP-MS
13	2.69	0.54	IAEA-436	ICP-MS

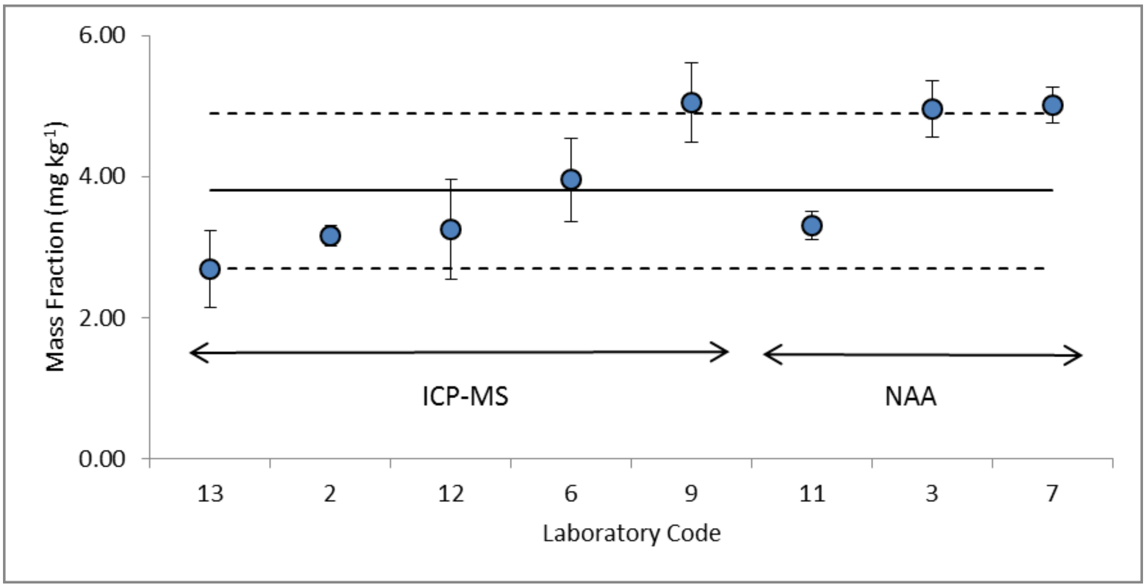

FIG. 23. Laboratory results for chromium mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 13. COPPER: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	2.38	0.23	DORM 3	ICP-MS
5	2.62	0.28	IAEA 407	ET-AAS
6	2.32	0.35	DORM-2	ICP-MS
8	2.25	0.18	IAEA436	ICP-MS
9	2.30	0.71	TORT-3	ICP-MS
12	1.87	0.46	NIST1566b	ICP-MS
13	2.49	0.50	IAEA-436	ICP-MS
IAEA	2.55	0.36	IAEA 407	ET-AAS
IAEA	2.72	0.33	IAEA 407	ICP-MS
IAEA	2.28	0.08		ID-ICP-MS

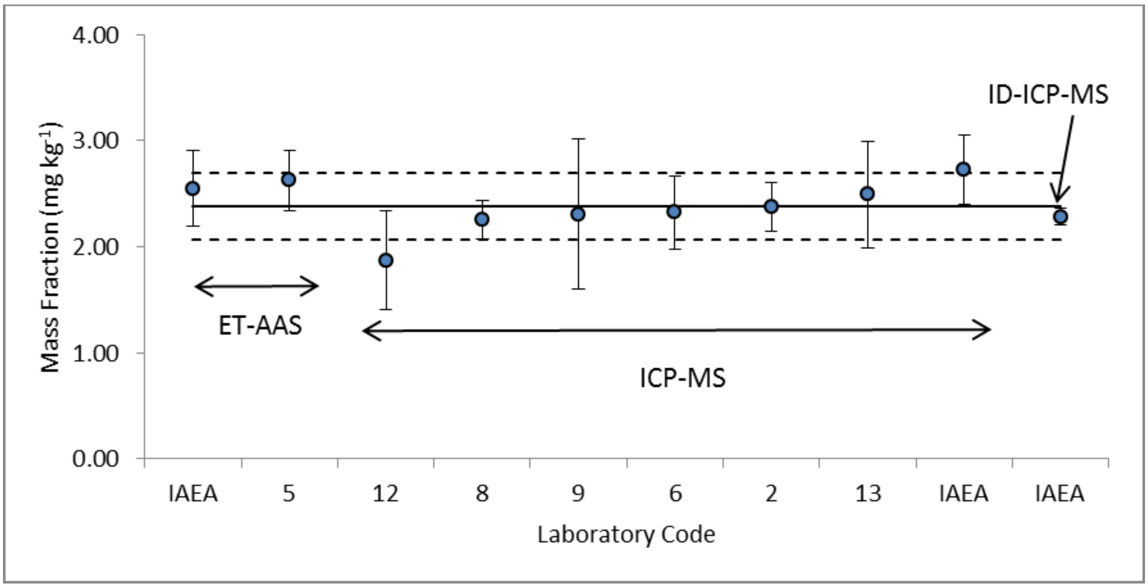

FIG. 24. Laboratory results for copper mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 14. IRON: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	122	8	DORM 3	ICP-OES
3	151	12	NIST SRM-1547	NAA
6	143	21	DORM-2	ICP-MS
7	149	18	SRM 1566b	NAA
9	139	11	TORT-3	ICP-MS
11	148	6	NIST 1566b	NAA
12	125	28	NIST1566b	ICP-MS
13	127	36	IAEA-436	ICP-OES
IAEA	149	21	IAEA 407	F-AAS

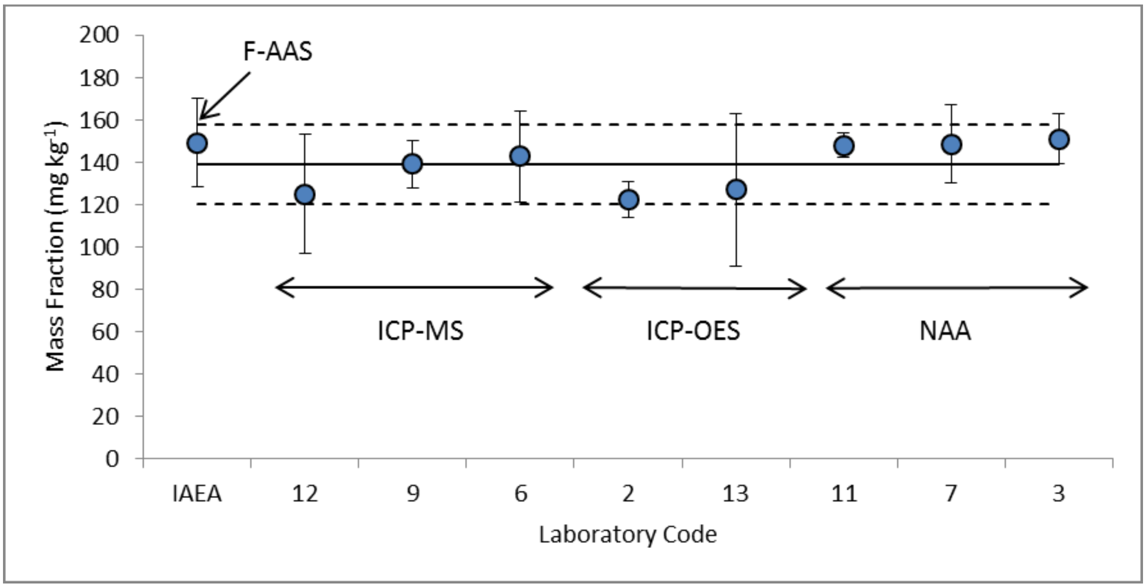

FIG. 25. Laboratory results for iron mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 15. TOTAL MERCURY: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	0.595	0.052	IAEA407	GC-ICPMS
1	0.605	0.016	IAEA407	ID-ICP-MS
3	0.572	0.044	DORM-4/BCR-463	CV-AAS
6	0.534	0.107	DORM-3	ICP-MS
8	0.527	0.038	IAEA407	ICP-MS
9	0.564	0.072	TORT-3	CV-AFS
10	0.600	0.102	DORM-4	Solid -AAS
11	0.637	0.076	DORM-4	CV-AAS
IAEA	0.568	0.024		ID-ICP-MS
IAEA	0.582	0.070	SRM 2703	Solid -AAS

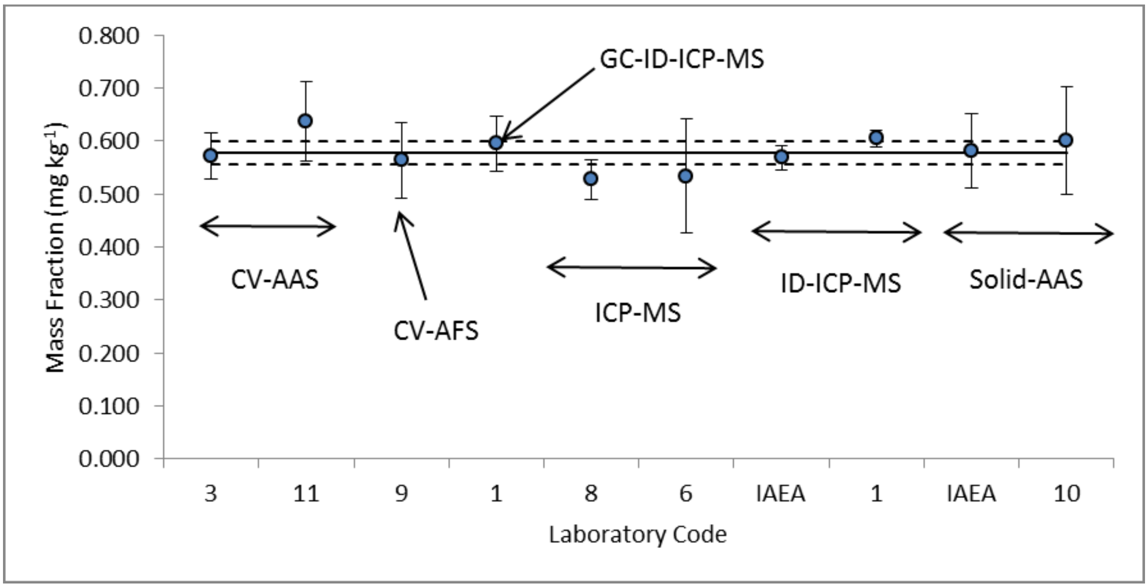

FIG. 26. Laboratory results for total mercury mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 16. METHYL MERCURY: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1} as Hg)

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	0.541	0.051	IAEA407	GC-ICPMS
3	0.537	0.026	BCR-463	CV-AFS
9	0.503	0.041	TORT-3	CV-AFS
10	0.562	0.073	DORM-4	CV-AFS
12	0.490	0.012	NRC DORM4	GC-AFS
IAEA	0.499	0.070	IAEA 407	GC-AFS
IAEA	0.530	0.024		ID-ICP-MS

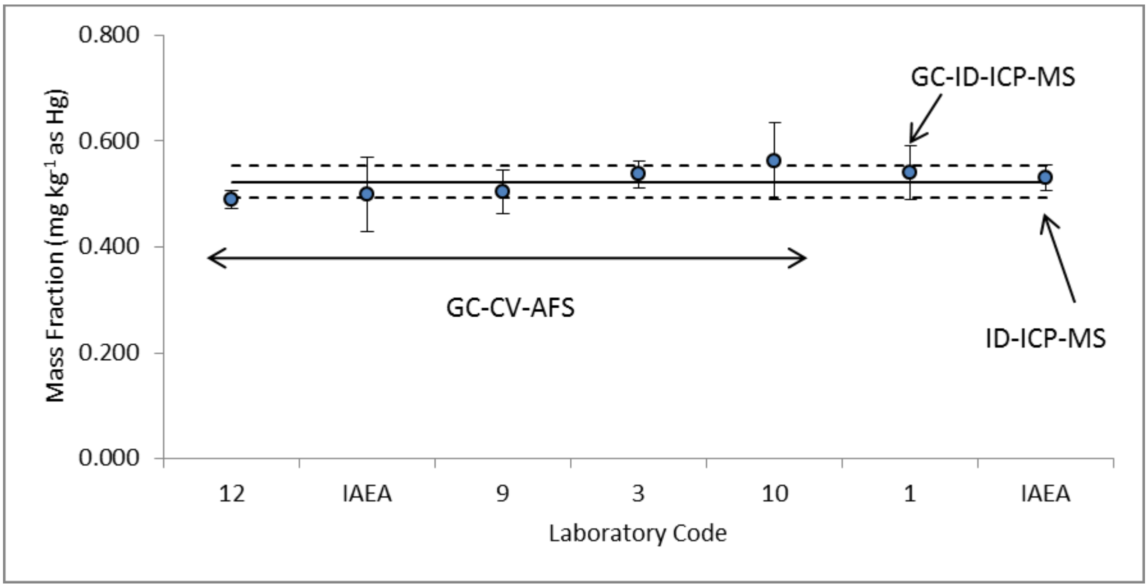

FIG. 27. Laboratory results for methyl mercury mass fraction (mg kg^{-1} as Hg) in IAEA-476.

TABLE 17. MANGANESE: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	17.6	1.0	DORM 3	ICP-MS
5	17.0	0.7	NIST 1566b	F-AAS
6	16.7	1.7	DORM-2	ICP-MS
8	16.2	1.1	IAEA407	ICP-MS
9	17.5	4.1	TORT-3	ICP-MS
11	16.1	1.0	NIST 1566b	NAA
12	15.6	3.4	NIST1566b	ICP-MS
13	14.0	4.0	IAEA-436	ICP-MS
IAEA	18.3	3.3	IAEA 407	ET-AAS
IAEA	15.5	2.2	SRM 2976	F-AAS

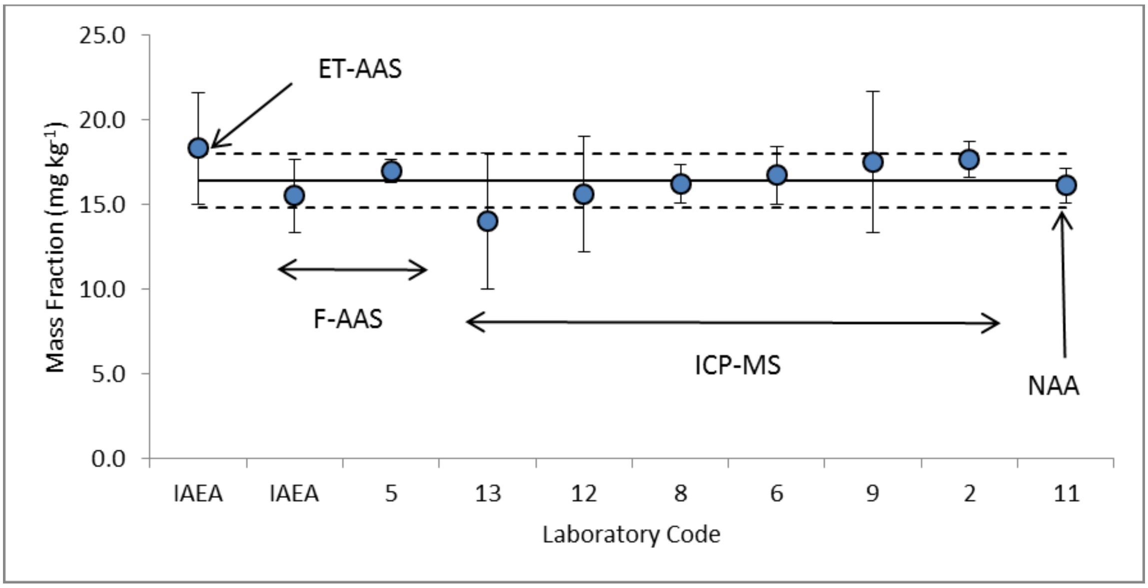

FIG. 28. Laboratory results for manganese mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 18. NICKEL: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	3.85	0.36	DORM 3	ICP-MS
6	4.56	0.68	DORM-2	ICP-MS
9	5.10	0.72	TORT-3	ICP-MS
12	3.72	0.89	NIST1566b	ICP-MS
13	3.63	1.26	IAEA-436	ICP-MS
IAEA	4.9	0.1		ID-ICP-MS

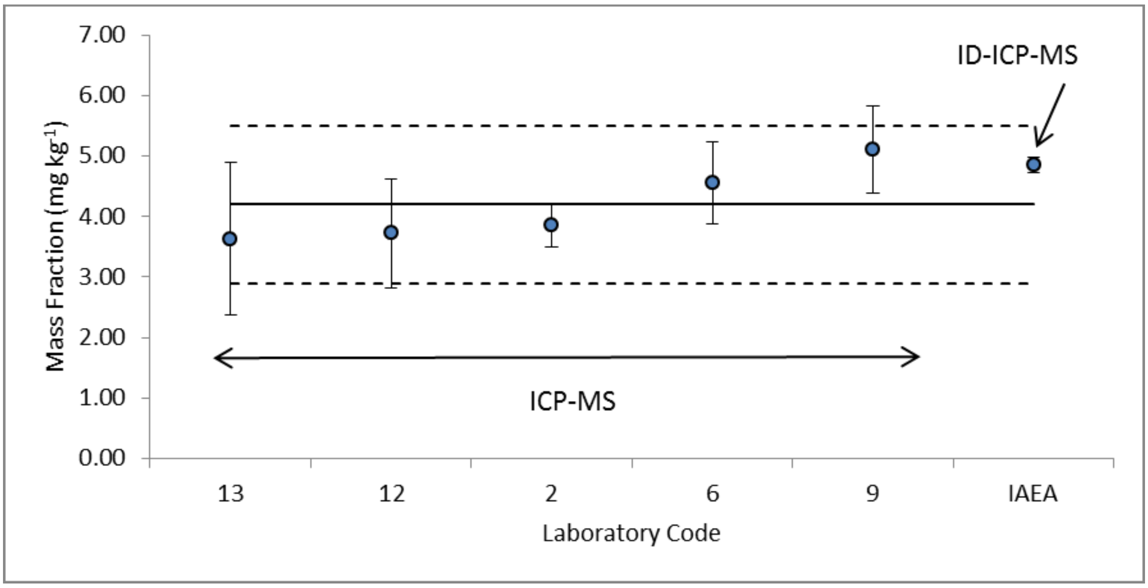

FIG. 29. Laboratory results for nickel mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 19. LEAD: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	0.676	0.084	DORM 3	ICP-MS
5	0.533	0.037	NIST 1566b	ET-AAS
6	0.660	0.132	DORM-2	ICP-MS
8	0.725	0.044	IAEA407	ICP-MS
9	0.704	0.043	TORT-3	ICP-MS
12	0.558	0.138	NIST1566b	ICP-MS
13	0.621	0.156	IAEA-461	ICP-MS
IAEA	0.637	0.115	IAEA 407	ET-AAS
IAEA	0.669	0.080	IAEA 407	ICP-MS
IAEA	0.657	0.023		ID-ICP-MS

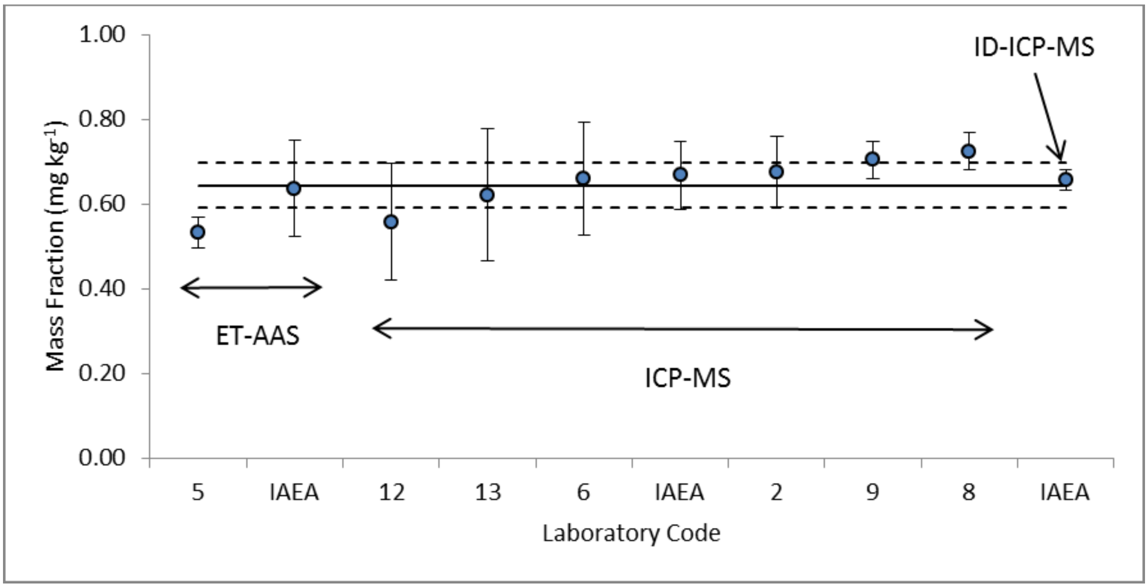

FIG. 30. Laboratory results for lead mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 20. SELENIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	2.15	0.20	DORM 3	ICP-MS
3	2.27	0.18	NIST SRM-1547	NAA
5	1.99	0.05	NIST 1566b	Hyd-AAS
6	2.19	0.44	DORM-2	ICP-MS
7	2.20	0.12	SRM 1566b	NAA
8	2.09	0.44	IAEA436	ICP-MS
9	2.05	0.07	TORT-3	ICP-MS
11	2.20	0.40	NIST 1566b	NAA
12	1.97	0.42	NIST1566b	ICP-MS
IAEA	2.12	0.30	IAEA 407	ICP-MS

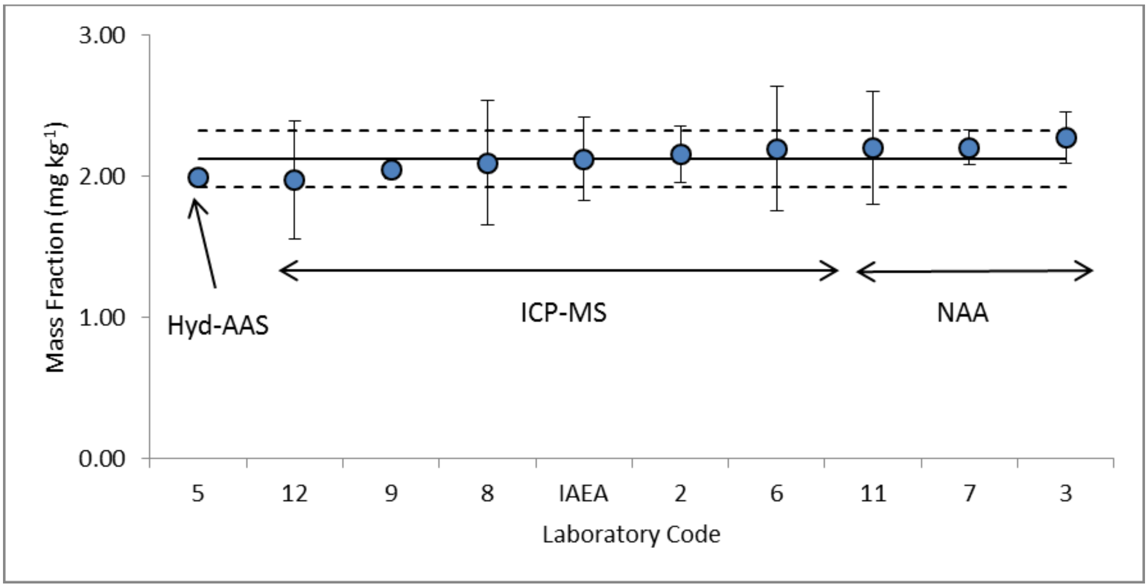

FIG. 31. Laboratory results for selenium mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 21. STRONTIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	147	17		ICP-OES
3	160	18	NIST SRM-1547	NAA
6	145	22	TORT-3	ICP-MS
7	154	8	SRM 1566b	NAA
8	151	8	IAEA407	ICP-MS
9	151	11	TORT-3	ICP-MS
12	128	38	NIST1566b	ICP-MS
13	128	38	IAEA-436	ICP-MS
IAEA	146	20	IAEA 407	F-AAS
IAEA	124	17	IAEA 407	ICP-MS

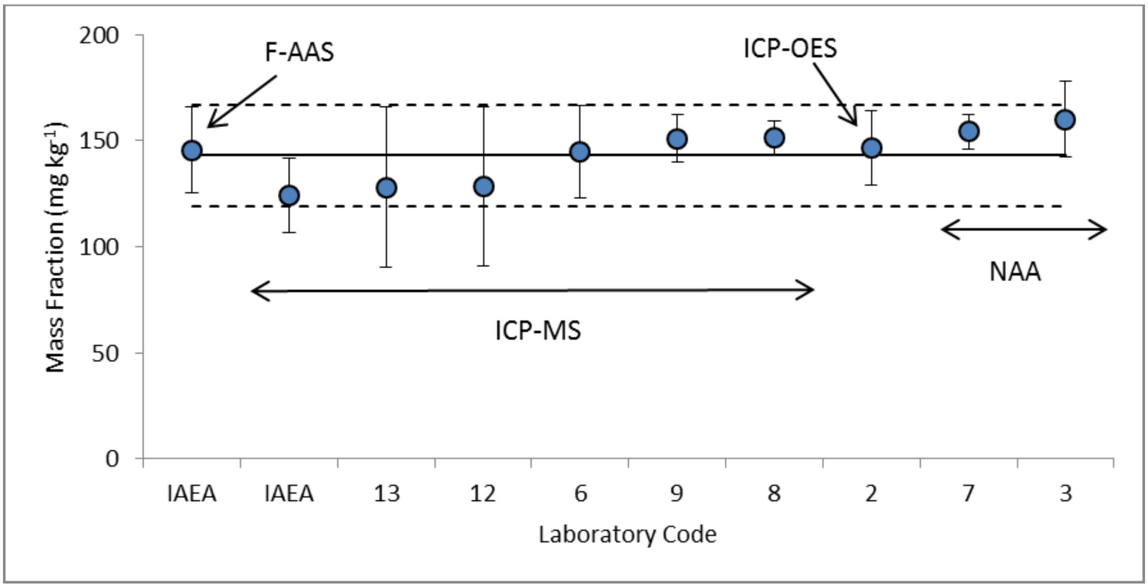

FIG. 32. Laboratory results for strontium mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 22. VANADIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
6	0.408	0.082	TORT-3	ICP-MS
9	0.280	0.035	TORT-3	ICP-MS
13	0.254	0.064	IAEA-461	ICP-MS
IAEA	0.430	0.060	IAEA 407	ICP-MS

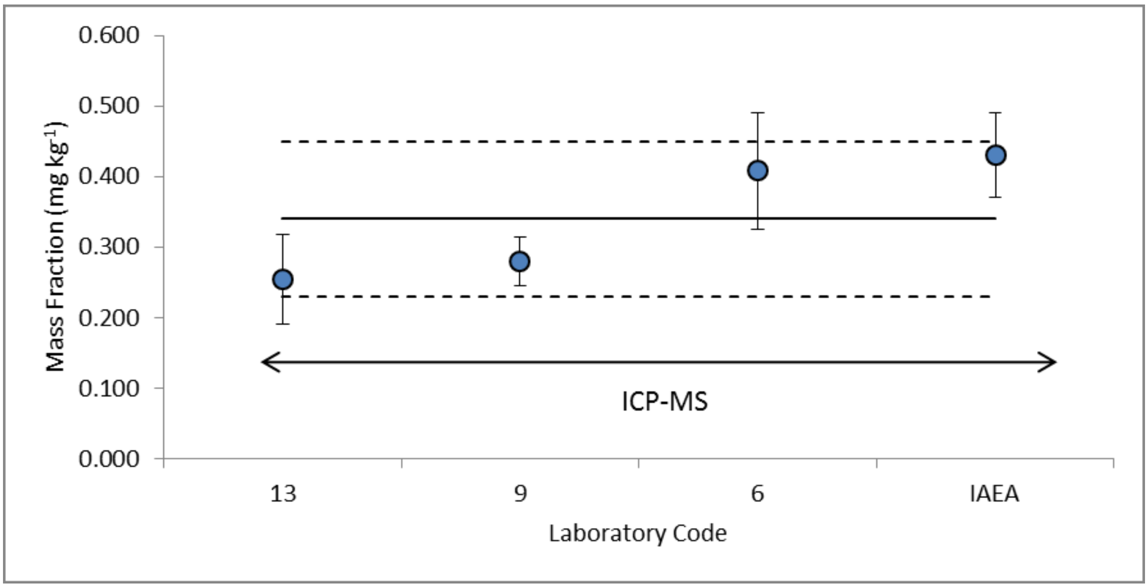

FIG. 33. Laboratory results for vanadium mass fraction (mg kg^{-1}) in IAEA-476.

TABLE 23. ZINC: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	51.6	2.2	DORM 3	ICP-OES
3	55.0	4.0	NIST SRM-1547	NAA
5	53.1	2.1	IAEA 407	F-AAS
6	56.4	8.5	DORM-2	ICP-MS
7	55.3	2.7	SRM 1566b	NAA
8	51.8	3.2	IAEA436	ICP-MS
9	50.2	2.4	TORT-3	ICP-MS
11	62.0	2.0	NIST 1566b	NAA
13	52.0	10.0	IAEA-436	ICP-MS
IAEA	52.1	7.3	IAEA 407	F-AAS
IAEA	53.0	7.4	IAEA 407	ICP-MS
IAEA	51.9	2.0		ID-ICP-MS

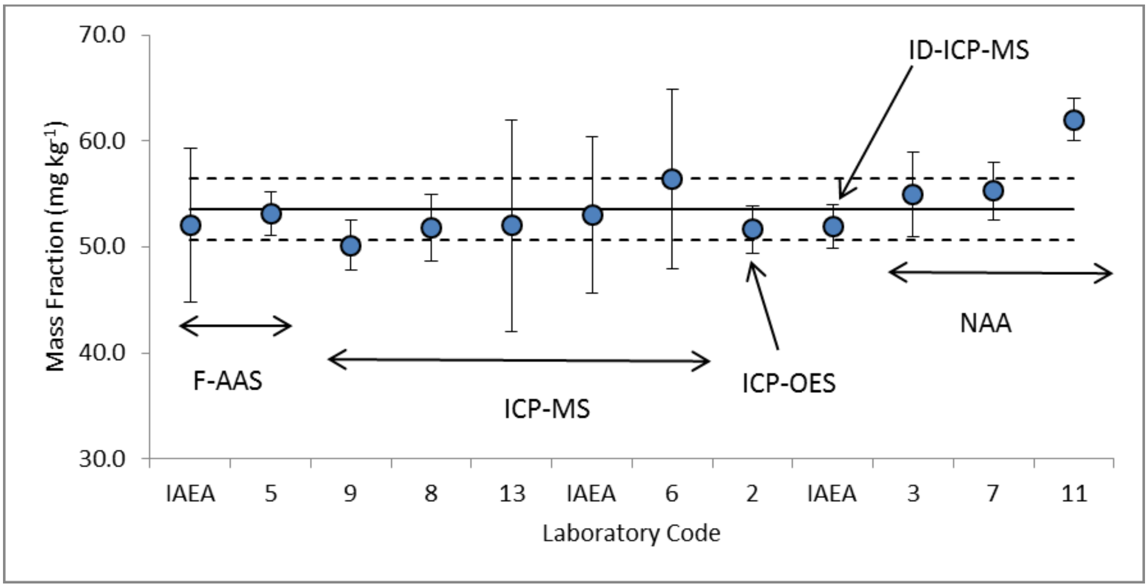

FIG. 34. Laboratory results for zinc mass fraction (mg kg^{-1}) in IAEA-476.

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