IAEA/AQ/68

IAEA Analytical Quality in Nuclear Applications Series No. 68

Certification of Trace Elements and Methylmercury Mass Fractions in Fish Flesh Homogenate IAEA-407A

IAEA
International Atomic Energy Agency

CERTIFICATION OF TRACE ELEMENTS AND METHYLMERCURY MASS FRACTIONS IN FISH FLESH HOMOGENATE IAEA-407A

The following States are Members of the International Atomic Energy Agency:

AFGHANISTAN
ALBANIA
ALGERIA
ANGOLA
ANTIGUA AND BARBUDA
ARGENTINA
ARMENIA
AUSTRALIA
AUSTRIA
AZERBAIJAN
BAHAMAS
BAHRAIN
BANGLADESH
BARBADOS
BELARUS
BELGIUM
BELIZE
BENIN
BOLIVIA, PLURINATIONAL STATE OF
BOSNIA AND HERZEGOVINA
BOTSWANA
BRAZIL
BRUNEI DARUSSALAM
BULGARIA
BURKINA FASO
BURUNDI
CAMBODIA
CAMEROON
CANADA
CENTRAL AFRICAN REPUBLIC
CHAD
CHILE
CHINA
COLOMBIA
COMOROS
CONGO
COSTA RICA
CÔTE D’IVOIRE
CROATIA
CUBA
CYPRUS
CZECH REPUBLIC
DEMOCRATIC REPUBLIC OF THE CONGO
DENMARK
DJIBOUTI
DOMINICA
DOMINICAN REPUBLIC
ECUADOR
EGYPT
EL SALVADOR
ERITREA
ESTONIA
ESWATINI
ETHIOPIA
FIJI
FINLAND
FRANCE
GABON
GEORGIA
GERMANY
GHANA
GREECE
GRENADA
GUATEMALA
GUYANA
HAITI
HOLY SEE
HONDURAS
HUNGARY
ICELAND
INDIA
INDONESIA
IRAN, ISLAMIC REPUBLIC OF
IRAQ
IRELAND
ISRAEL
ITALY
JAMAICA
JAPAN
JORDAN
KAZAKHSTAN
KENYA
KOREA, REPUBLIC OF
KUWAIT
KYRGYZSTAN
LAO PEOPLE’S DEMOCRATIC REPUBLIC
LATVIA
LEBANON
LESOTHO
LIBERIA
LIBYA
LIECHTENSTEIN
LITHUANIA
LUXEMBOURG
MADAGASCAR
MALAWI
MALAYSIA
MALI
MALTA
MARSHALL ISLANDS
MAURITANIA
MAURITIUS
MEXICO
MONACO
MONGOLIA
MONTENEGRO
MOROCCO
MOZAMBIQUE
MYANMAR
NAMIBIA
NEPAL
NETHERLANDS
NEW ZEALAND
NICARAGUA
NIGER
NIGERIA
NORTH MACEDONIA
NORWAY
OMAN
PAKISTAN
PALAU
PANAMA
PAPUA NEW GUINEA
PARAGUAY
PERU
PHILIPPINES
POLAND
PORTUGAL
QATAR
REPUBLIC OF MOLDOVA
ROMANIA
RUSSIAN FEDERATION
RWANDA
SAINT KITTS AND NEVIS
SAINT LUCIA
SAINT VINCENT AND THE GRENADINES
SAMOA
SAN MARINO
SAUDI ARABIA
SENEGAL
SERBIA
SEYCHELLES
SIERRA LEONE
SINGAPORE
SLOVAKIA
SLOVENIA
SOUTH AFRICA
SPAIN
SRI LANKA
SUDAN
SWEDEN
SWITZERLAND
SYRIAN ARAB REPUBLIC
TAJIKISTAN
THAILAND
TOGO
TONGA
TRINIDAD AND TOBAGO
TUNISIA
TÜRKİYE
TURKMENISTAN
UGANDA
UKRAINE
UNITED ARAB EMIRATES
UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND
UNITED REPUBLIC OF TANZANIA
UNITED STATES OF AMERICA
URUGUAY
UZBEKISTAN
VANUATU
VENEZUELA, BOLIVARIAN REPUBLIC OF
VIET NAM
YEMEN
ZAMBIA
ZIMBABWE

The Agency’s Statute was approved on 23 October 1956 by the Conference on the Statute of the IAEA held at United Nations Headquarters, New York; it entered into force on 29 July 1957. The Headquarters of the Agency are situated in Vienna. Its principal objective is “to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world”.

IAEA/AQ/68

IAEA Analytical Quality in Nuclear Applications Series No. 68

CERTIFICATION OF TRACE ELEMENTS AND METHYLMERCURY MASS FRACTIONS IN FISH FLESH HOMOGENATE IAEA-407A

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 2022

COPYRIGHT NOTICE

Marketing and Sales Unit, Publishing Section
International Atomic Energy Agency
Vienna International Centre
PO Box 100
1400 Vienna, Austria
fax: +43 1 26007 22529
tel.: +43 1 2600 22417
email: sales.publications@iaea.org
www.iaea.org/publications

For further information on this publication, please contact:

Marine Environmental Studies Laboratory
IAEA Environment Laboratories
International Atomic Energy Agency
4a Quai Antoine 1er, 98000
Principality of Monaco

CERTIFICATION OF TRACE ELEMENTS AND METHYLMERCURY MASS FRACTIONS
IN FISH FLESH HOMOGENATE IAEA-407A
IAEA, VIENNA, 2022
IAEA/AQ/68
ISSN 2074–7659

Printed by the IAEA in Austria
November 2022

FOREWORD

Monitoring contaminants in the marine environment is a prerequisite when developing accurate environmental assessments and evaluating the effectiveness of pollution control. Monitoring can only be valid if the results, obtained in different places and at different times, are comparable. Comparing environmental data is only achievable when results can be traced to a common system of reference. In this respect certified reference material plays a major role. To support Member States in their monitoring activities, the IAEA produces certified reference materials that are characterized for trace elements and methylmercury mass fractions in samples of marine-origin biota and sediments.

This publication describes the production of certified reference material IAEA-407A which is based on a new characterization study of existing reference material IAEA-407. In 2003 the IAEA produced certified reference material IAEA-407 which has been characterized for trace elements and methylmercury mass fractions in fish homogenate.

IAEA-407A was produced following the requirements of the international standard ISO 17034 and ISO Guide 35, both of which relate to the production of certified reference material. Analytical laboratories with demonstrated measurement competence participated in characterizing the material.

The IAEA is grateful to the Government of Monaco for its support and wishes to thank the experts and laboratories involved for their contributions. The IAEA officers responsible for this publication were S. Azemard and E. Vasileva of the Marine Environment Laboratories.

EDITORIAL NOTE

This publication has been prepared from the original material as submitted by the contributors and has not been edited by the editorial staff of the IAEA. The views expressed remain the responsibility of the contributors and do not necessarily reflect those of the IAEA or the governments of its Member States.

Neither the IAEA nor its Member States assume any responsibility for consequences which may arise from the use of this publication. This publication does not address questions of responsibility, legal or otherwise, for acts or omissions on the part of any person.

The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

The mention of names of specific companies or products (whether or not indicated as registered) does not imply any intention to infringe proprietary rights, nor should it be construed as an endorsement or recommendation on the part of the IAEA.

The IAEA has no responsibility for the persistence or accuracy of URLs for external or third party Internet web sites referred to in this publication and does not guarantee that any content on such web sites is, or will remain, accurate or appropriate.

CONTENTS

1. INTRODUCTION

1.1. BACKGROUND

Monitoring laboratories are involved in the production of local or regional environmental data used in environmental assessments. The comparability of data, used in monitoring studies, is of essential importance, even though they are produced at different places and times. If monitoring results are to be comparable, it is essential that they are based on reliable measurement standards whose values are linked to an internationally accepted system of units. This can be achieved using Certified Reference Materials (CRMs) with demonstrated traceability and stated uncertainty of reference values. CRMs are valuable tools for the performance evaluation of analytical techniques and for the validation of analytical methods used in environmental monitoring programmes.

The Marine Environmental Studies Laboratory (MESL) of the International Atomic Energy Agency's Environment Laboratories (IAEA-NAEL) provides assistance to Member State's laboratories to enhance the quality of analytical measurement results for trace elements and organic contaminants in marine samples. This is achieved through the production of CRMs, organisation of interlaboratory comparisons and proficiency tests, and by conducting training courses on the analysis of contaminants in marine samples.

1.2. OBJECTIVES

To support the accurate and traceable determination of trace element and methyl mercury in fish tissue in the frame of pollution and public health assessment, a new CRM, IAEA-407A, characterized for trace element and methylmercury mass fractions was produced.

The new CRM will assist laboratories in validation of their analytical methods and controlling the quality of produced analytical results for the determination of trace elements and methyl mercury in marine biota samples [1]. The CRM IAEA-407A will be also used for strengthening mercury monitoring efforts of the marine environment, in order to assess mercury contamination, as well as to control the efficiency of the measures undertaken, by the Member States adopting the Minamata convention [2].

1.3. SCOPE

The scope of this publication is to describe the re-certification process for trace elements and methylmercury mass fractions in fish homogenate sample. The new CRM IAEA-407A is produced according to the requirements of the ISO 17034 standard [3] and can be used in laboratory practice for quality control and method validation purposes. It has been produced to satisfy the demands of laboratories dealing with environmental and food safety analyses.

1.4. STRUCTURE

This publication is structured in five sections, first being the Introduction. The second section provides the methodology used for the preparation of the reference material, including the selection of laboratories for the characterization campaign, and all procedures for the homogeneity, stability, and reference values assignment. The section 3 covers the results and discussions on the determination and justifications of the assigned values and their expanded uncertainties. Then, section 4 provides the information on metrological traceability and commutability of the new CRM. Final section 5 provides the conclusions from the certification campaign of the IAEA 407A.

2. METHODOLOGY

2.1. PREPARATION OF THE MATERIAL

IAEA-407A was prepared by rebottling 170 units of IAEA-407 into sealed and acid cleaned polypropylene containers. The content of each amber borosilicate bottle of IAEA-407 was divided into two plastic containers after manual shaking. More details about the preparation of IAEA-407, are given in reference [4]. Preliminary investigations of the within and between bottles homogeneity indicated that the starting material was homogeneous and didn't need additional homogenization before its rebottling.

2.2. SELECTION OF LABORATORIES

The selection of participants in the characterization study was based on the measurement performances demonstrated by laboratories in the previous 3 interlaboratory comparisons (ILCs), organized by the IAEA. Only results of laboratories providing reliable measurement results, having a quality system in place, using validated methods, and applying uncertainty and traceability concepts were invited and accepted for participation in the certification campaign [3].

The list of laboratories participating in the characterization study is presented on page 65.

2.3. HOMOGENEITY ASSESSMENT

A key requirement for any reference material, certified as a batch, is the equivalence between the various CRM units in this batch. In this respect, the important issue is whether the variation between units is significant compared to the uncertainty of the certified value. Consequently, ISO 17034 [3] requires reference material producers to quantify the between-unit variation.

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. In total, 12 bottles from the whole batch were selected, using random stratified sampling. Duplicate subsamples from each bottle were analyzed for their total element and methyl mercury mass fractions in the inorganic chemistry laboratories of MESL.

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

ANOVA allows the calculation of within-unit standard deviation s_{wb} and the between-units standard deviation s_{bb}:

$$s_{\mathrm{wb}} = u_{\mathrm{wb}} = \sqrt{MS_{\mathrm{wb}}} \tag{1}$$

$$s_{\mathrm{bb}} = u_{\mathrm{bb}} = \sqrt{\frac{MS_{\mathrm{bb}} - MS_{\mathrm{wb}}}{n}} \tag{2}$$

n is the number of replicates sub-samples per bottle and $\nu_{MS\mathrm{wb}}$ is the degrees of freedom of MS_{wb}.

s_{bb} and s_{wb} are estimates of the standard deviations and are subject to random fluctuations, therefore, the mean square between groups (MS_{bb}) can be smaller than the mean square within groups (MS_{wb}), 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. [5]. 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.

$$u^*_{\mathrm{bb}} = \sqrt{\frac{s_{\mathrm{wb}}}{n}} \sqrt[4]{\frac{2}{\nu_{MS\mathrm{wb}}}} \tag{3}$$

The within-unit inhomogeneity does not influence the uncertainty of the certified value, when the minimum sample intake is respected. Therefore, the determination of the minimum size of an aliquot that is representative for the whole unit is an important step in the characterization process. Quantification of within-unit (in this case: within-bottles) inhomogeneity is, therefore, also necessary for the determination of the minimum sample intake.

ISO Guide 35 [6] recommends, this type of assessment when the minimum sample intake is significantly less than the unit size. In total each of the 6 subsamples from the same sample unit was analyzed in triplicates

The quantification of uncertainty arising from within-unit homogeneity was done also by ANOVA which in this case can separate within-bottle variation (Eq 5.) from the analysis variation applying (Eq.4). The between-group mean square (MS_{wb}) represents the within bottle variance while the within-group mean square (MS_{method}) represents the analytical variation. For total Hg, as only one measurement can be performed per subsample, s_{method} is obtained from validation and is estimated as 2.3%.

$$s_{method} = \sqrt{MS_{method}} \tag{4}$$

$$s_{wb} = \sqrt{\frac{MS_{wb}-MS_{metho}}{n}} \tag{5}$$

The significance of within-bottle inhomogeneity was evaluated using an F statistical test and was found significant for some analytes. If within-bottle inhomogeneity was significant, the uncertainty u_{hom} associated to homogeneity of the material at the prescribed minimum sample size was estimated by Eq. 6, if not u_{hom} was estimated following Eq. 7.

$$u_{ho} \quad = \sqrt{u_{wb}^2 + u_{bb}^2} \tag{6}$$

$$u_{hom} = u_{bb} \tag{7}$$

The between- and within-unit homogeneities were evaluated by the determination of Ag, Al, As, Ca, Cd, Co, Cr, Cu, K, Li, Mg, Mn, Ni, Pb, Rb, Sb, Se, Sn, Sr, V and Zn mass fractions in the subsamples, selected for the respective study. For all analytes except for Hg and methyl mercury (MeHg), subsamples of 0.2 g were mineralized with 5 ml concentrated nitric acid (Optima Grade, Fisher, Switzerland) and 2ml of hydrogen peroxide (Primary trace metal grade, Fisher, Switzerland) in a microwave oven, applying a preliminary validated sample digestion program [7]. The final measurements were performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (X Series 2 from Thermo Fisher, Germany) for Ag, Al, As, Cd, Co, Cr, Cu, Li, Mn, Ni, Pb, Sb, Se, Sn, Sr, and V or Flame Atomic Absorption Spectrometry (AAS) (Contra 6 from Analytik Jena, Germany) for Ca, K, Mg, Rb and Zn under repeatability conditions, and in a randomized way. The determination of total Hg was done in solid subsamples (50 mg) with a solid mercury analyzer (DMA, Milestone, USA). Methylmercury determination was performed by Gas Chromatography coupled to Atomic Fluorescence Spectroscopy (GC-AFS) (Merx, Brooks Rand, USA) after acidic leaching in microwave oven of 0.2 g as described in [8].

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

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

2.4. STABILITY STUDY

Stability tests are necessary for the establishment of conditions for storage (long term stability) as well as conditions for the transportation of the units to the customers (short term stability).

The stability studies were carried out using an isochronous design [9] . In this approach, samples are stored for a given time at different temperature conditions. Afterwards, the samples are moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples were analysed under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions significantly improves the sensitivity of the stability tests.

Original material IAEA-407 has been prepared and bottled in 2000, at the time of bottling some bottles (10) have been stored under so called “reference” condition: -20 ± 2 °C in the dark. The other produced units were then stored under “normal” conditions: +20 ± 5 C° in the dark. To evaluate potential degradation of the material over 21 years, 3 bottles stored under “reference” conditions and 9 bottles stored under “normal” conditions were randomly selected. Two subsamples from each bottle were analysed for their total element mass fractions, as described in Section 2.3.

In order to evaluate potential degradation of the material during the transportation, some bottles were stored at (60 ± 3) °C. Two bottles were selected after 1, 2 and 3 weeks, respectively, and placed under “reference” condition (-20 ± 2 °C). Duplicate subsamples from each bottle were analysed for their total element and methyl mercury mass fractions as describe in Section 2.3.

The measurements were performed under repeatability conditions and in randomized way in order to be able to separate a potential analytical drift from a trend over the storage time. The results were corrected for the water content, determined in each unit by using the protocol described in the Section 2.6. Based on experience with similar matrix, no degradation was expected; for this reason, units used for long- and short-term stability were also used for the homogeneity test to optimize the number of analyses.

2.5. CHARACTERIZATION

The fish sample was initially analysed in the MESL' Inorganic chemistry laboratories 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 candidate CRM, accompanied by an information sheet and a reporting form. Participants were requested to analyse Ag, Al, As, Ca, Cd, Co, Cr, Cu, Hg, K, Li, MeHg, Mg, Mn, Ni, Pb, Rb, Sb, Se, Sn, Sr, V, and Zn using validated analytical methods. They were asked to report measurement results (three replicates and average value), expanded uncertainty, information on the applied quality control procedures as well as the information on the standard calibration solutions used in the measurement step.

In addition, each participant has received 1 bottle of IAEA-476 (CRM with similar matrix composition to the test sample) as a blind quality control sample. For Ag, As, Cd, Cu, Hg, MeHg, Mn, Pb, Se and Zn the reported results for the blind quality control sample were evaluated against the certified values and associated uncertainties of the CRM IAEA-476 [10]. For analytes not characterized in IAEA-476 (Ca, K, Mg, Sr and V) the reported results for the IAEA 476 CRM were evaluated against the assigned values obtained in the interlaboratory comparison exercise organized with the CRM IAEA-476 as a test sample [11].

Laboratories were requested to provide moisture corrected results. As the moisture content in the sample is an operationally dependent parameter, the method for moisture determination was preliminary prescribed to all participating laboratories.

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

TABLE 1. DATASET EXCLUDED FROM THE FURTHER EVALUATION AFTER TECHNICAL REVIEW

Lab code	Analyte	Description
4	As, Cd, Cu, Mn, Zn	QC result does not agree within uncertainties
6	As, Pb	QC result does not agree within uncertainties
7	As, Ca, Hg, Pb, Sr, V, Zn	QC result does not agree within uncertainties
10	As, Mn, Se	QC result does not agree within uncertainties
12	Ag, Pb	QC result does not agree within uncertainties
6	As	High uncertainty (>35%)
12	Ag, Pb	Results too close to quantification limit (QL)

The characterization of the trace elements mass fractions in the sample was based on the application of several analytical techniques. The applied analytical techniques in this characterization study are summarized in Figure 1. The abbreviations of the applied analytical techniques are given in Table 2.

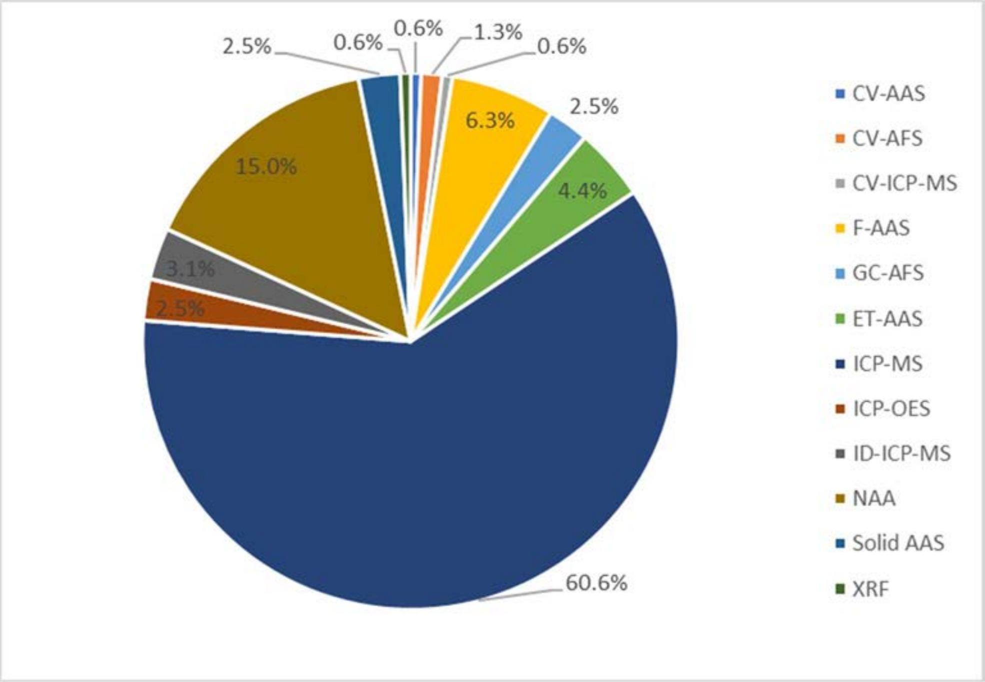

FIG. 1. Analytical methods used for the characterization of trace element mass fractions in IAEA-407A.

TABLE 2. ABBREVIATIONS USED FOR ANALYTICAL TECHNIQUES

Abbreviation	
AAS	Atomic Absorption Spectrometry
AFS	Atomic Fluorescence Spectrometry
CV	Cold Vapor
ET-AAS	Electro Thermal Atomic Absorption Spectrometry
F-AAS	Flame Atomic Absorption Spectrometry
GC	Gaz chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ID	Isotope Dilution
NAA	Neutron Activation Analysis (or instrumental neutron activation)
XRF	X ray Fluorescence

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 of the moisture was developed at the IAEA and prescribed to participants in the characterization study. The drying procedure at 85±2°C was established after experimental evaluation of sample stability.

Correction for dry mass was obtained from separate portions of the material at minimum sample mass of 0.5 g. The weighing and repeated drying were performed until constant mass was attained (generally 24 hours). Moisture, determined at 85°C (10 subsamples from 5 bottles) was found to be (5.1±0.5) % for bottles kept at 20°C.

3. RESULTS AND DISCUSSION

3.1. RESULTS OF HOMOGENEITY STUDY

3.1.1. Between-unit homogeneity

Twelve units of the candidate CRM sample were selected by using a random stratified sample picking scheme and analysed for their trace elements and MeHg contents in a duplicate.

Regression analyses were performed to evaluate potential trends in the analytical and processing sequences. Significant analytical sequence trend was found for Ag, As and Li. A linear regression model was chosen as a reasonable approximation. Before other statistical treatments, the mass fraction results for Ag, As and Li were corrected following Eq. 8.

$$\text{Corrected result} = \text{Measured Result} - (b \times i) \tag{8}$$

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

For Pb a significant processing trend was observed; in that case the estimate of heterogeneity between bottles was modeled as the half width of a rectangular distribution following Eq.9.

$$u_{rect} = \frac{highe \quad result - lowe \quad result}{2\sqrt{3}} \tag{9}$$

Grubbs and Dixon tests at 95% and 99% confidence levels were performed to identify potential outlying individual results or bottle means. Individual outliers were detected for As, Cd, K, Ni, Pb and Sn. The outliers detected for Cd, Ni, Pb and Sn were linked to contamination during the analytical run and were rejected before the rest of statistical treatment. To keep balanced datasets (i.e., two results per units) the results from both duplicates of the unit were rejected. In the case of As and K, since no technical reasons were found for observed single outliers, and distributions were normal, all results were retained.

For Cd and Mg one bottle mean was detected as an outlier, between bottle heterogeneity was modeled as the rectangular distribution limited by the outlying average using Eq.10.

$$u_{rect} = \frac{|outlier - y|}{\sqrt{3}} \tag{10}$$

where y is the average of all results.

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 the data set has a single mode of distribution,

For the elements for which ANOVA was applied the uncertainty contributions due to the between-unit homogeneity were estimated according to ISO Guide 35 [6] as the maximum values obtained with Eq. 2 or Eq. 3 and presented in Table 3.

TABLE 3. ESTIMATES OF BETWEEN-BOTTLE HOMOGENEITY CONTRIBUTIONS TO THE TOTAL UNCERTAINTY OF THE ASSIGNED TRACE ELEMENTS MASS FRACTION

	s_{bb}	u^*_{bb}	u_{rect}	u_{bb}
Ag	<0	2.0%	-	2.0%
Al	<0	4.7%	-	4.7%
As	1.2%	0.6%	-	1.2%
Ca	3.3%	1.6%	-	3.3%
Cd	1.4%	2.0%	5.4%	5.4%
Co	2.5%	1.9%	-	2.5%
Cr	<0	5.6%	-	5.6%
Cu	<0	1.0%	-	1.0%
Hg	<0	0.7%	-	0.7%
K	<0	1.0%	-	1.0%
Li	1.8%	1.1%	-	1.8%
MeHg	0.9%	1.6%	-	1.6%
Mg	1.9%	0.9%	3.5%	3.5%
Mn	5.1%	1.3%	-	5.1%
Na	1.4%	0.5%	-	1.4%
Ni	<0	5.0%	-	5.0%
Pb	7.0%	4.0%	7.0%	7.0%
Rb	<0	4.0%	-	4.0%
Sb	5.0%	4.0%	-	5.0%
Se	2.0%	1.0%	-	1.0%
Sn	2.4%	6.0%	-	6.0%
Sr	3.8%	1.7%	-	3.8%
V	2.1%	1.3%	-	2.1%
Zn	1.0%	1.2%	-	1.2%

3.1.2. Within-unit homogeneity

For the within-bottle study, 6 sub samples from 1 unit were decomposed as already described in (2.3) and each of the obtained solutions measured 3 times, using a randomized scheme. Regression analyses were performed to evaluate potential trends in the analytical run. Significant analytical sequence trend was found for Cd, Cr and Rb. A linear regression model was chosen as a reasonable approximation. Before other statistical treatment. results were corrected following Eq. 8.

Grubbs and Dixon tests at 95% and 99% confidence levels were performed to identify potentially outlying individual results. Outliers were detected for Cd, Co, Cu, Ni, Pb and Sn due to contamination and were rejected before continuing statistical evaluation of results.

As a prerequisite for the application of ANOVA for the estimation of uncertainty arising from within unit homogeneity, it was verified whether the individual results follow an approximately normal distribution and are unimodally distributed. All retain data were normally distributed.

Table 4 is summarizing all results from the homogeneity study.

TABLE 4. ESTIMATES OF INHOMOGENEITY CONTRIBUTIONS TO THE TOTAL UNCERTAINTY FOR THE ASSIGNED TRACE ELEMENT MASS FRACTIONS

Analyte	Significance	s_{method}	u_{wb}	u_{bb}	u_{hom}
Ag	Yes (95, 99%)	1.7%	9.5%	2.0%	9.7%
Al	Yes (95, 99%)	4.6%	12.7%	4.7%	13.5%
As	no	1.7%	0.3%	1.2%	1.2%
Ca	no	2.6%	0.7%	3.3%	3.3%
Cd	no	1.4%	0.1%	5.4%	5.4%
Co	Yes (95, 99%)	3.7%	6.8%	2.5%	7.2%
Cr	Yes (95, 99%)	14.0%	11.0%	5.6%	12.3%
Cu	no	5.1%	4.7%	1.0%	1.0%
Hg	no	2.3%	0.7%	0.7%	0.7%
K	Yes (95%)	3.5%	1.8%	1.0%	2.1%
Li	no	4.7%	<0	1.8%	1.8%
MeHg	no	3.2%	0.9%	1.6%	1.6%
Mg	Yes (95, 99%)	1.6%	1.3%	3.5%	3.7%
Mn	no	3.4%	2.9%	5.1%	5.1%
Na	Yes (95, 99%)	1.5%	2.6%	1.4%	3.0%
Ni	no	5.1%	1.7%	5.0%	5.0%
Pb	Yes (95, 99%)	0.8%	13.1%	7.0%	14.9%
Rb	no	5.8%	<0	4.0%	4.0%
Sb	Yes (95,99%)	3.4%	3.1%	5.0%	5.9%
Se	no	2.5%	<0	1.0%	1.0%
Sn	Yes (95,99%)	1.9%	16.6%	6.0%	17.7%
Sr	no	1.4%	1.2%	3.8%	3.8%
V	no	2.3%	1.7%	2.1%	2.1%
Zn	no	2.9%	0.9%	1.2%	1.2%

3.2. RESULTS FOR STABILITY STUDY

3.2.1. Long–term stability

The samples selected for long term stability study were analysed and each of the analytes was evaluated individually.

Evaluation of data was carried out by performing a linear regression on the determined mass fractions versus time. No significant slope at 99% level of confidence was detected for any of investigated trace elements in the long–term stability study at +20°C. The approach proposed in ISO Guide 35 [6] was followed, an uncertainty contribution related with the stability of the candidate reference material (u_{lt}) was estimated as the standard error of the slope multiplied by the selected shelf life of 5 years.

Results of the long–term stability study are displayed in the Appendix I (Figures 2 – 25) and in Table 5.

3.2.2. Short–term stability

Evaluation of data was carried out by performing a linear regression on the determined mass fractions versus time. For As, Ca, Se and Sr the slope was significant, as a result an uncertainty component for short term stability (u_{st}) was estimated multiplying the standard error of the slope by the estimated transport time of 2 weeks.

Results of the short–term stability study are displayed in the Appendix II (Figures 26 – 48) and in Table 5.

3.3. DETERMINATION OF ASSIGNED VALUES AND THEIR UNCERTAINTIES

The characterization campaign resulted in 4 to 10 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 (2.2).

The median and unweighted mean of the means were calculated and compared for each analyte. 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 the IAEA-407A CRM.

The uncertainties associated with the assigned values were calculated according to ISO Guide 35 [6]. The relative combined uncertainty of the certified value of the CRM consists of uncertainty contributors related to its characterization (u_{char}), sample heterogeneity (u_{hom}), long term stability (u_{lt}) and short term stability (u_{st}). These different contributions were combined and multiplied with a coverage factor *k* to estimate the expanded uncertainty using Eq. 11.

$$U = k \times \sqrt{u_{char}^2 + u_{lt}^2 + u_{st}^2 + u_{hom}^2} \tag{11}$$

Where

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

u_{hom} was estimated as described in section 3.1

u_{lt} was estimated as described in section 3.2

u_{st} was estimated as zero, or as described in section 3.2

u_{char} was estimated as described in ISO Guide 35 [6] using Eq. (12):

$$u_{char} = \frac{s}{\sqrt{p}} \tag{12}$$

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

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

Certified values were calculated as the mean of the means of accepted datasets for elements fulfilling the following criteria: at least 5 results from 2 methods were available and relative expanded uncertainties of the assigned value are less than 15%. The certified values are presented in Table 6, together with their expanded uncertainty.

The above conditions were not valid for Ag, al, Co, Cr, Li, Ni, Pb, Sb and Sn mass fractions, therefore they were provided as information values only. The information values for above mentioned elements are presented in Table 7.

The results for the mass fractions of the certified trace elements, as reported by the participants in this certification and grouped by methods, are presented in Appendix III. In Appendix IV is presented the information for the trace elements with information values. In all figures the reported results are plotted versus the assigned value denoted by a bold line, while the dashed lines represent the expanded uncertainty (k=2) associated with assigned value (as calculated with the Eq. 11). The error bars represent the expanded uncertainty as reported by participants.

As shown previously in Figure 1 and in Figures 49-63, methods with different quantification principals (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 characterization of the material.

TABLE 5. MEAN OF THE MEANS AND UNCERTAINTIES

Analyte	Mean of the means mg kg^{-1}	$u_{\mathrm{char,rel}}$ %	$u_{\mathrm{hom,rel}}$ %	$u_{\mathrm{st,rel}}$ %	$u_{\mathrm{lt,rel}}$ %	U_{rel} (k=2) %
Ag	0.035	4.6	9.7		0.7	21.5
Al	11.1	15.7	13.5		1.0	41.5
As	13.3	1.9	1.2	0.5	0.2	4.7
Ca	24.6×10^3	5.0	3.3	1.3	0.4	12.4
Cd	0.184	1.6	5.4		0.5	11.3
Co	0.088	7.3	7.2		0.6	20.5
Cr	0.672	8.3	12.3		1.5	29.9
Cu	3.09	3.2	1.0		0.3	6.7
Hg	0.219	2.3	0.7		0.2	4.8
K	12.8×10^3	3.1	2.1		0.3	7.4
Li	0.686	9.6	1.8		0.4	19.5
MeHg*	0.193	2.7	1.6		0.4	6.2
Mg	2.39×10^3	2.6	3.7		0.3	9.0
Mn	3.49	2.2	5.1		0.7	11.2
Na	14.2×10^3	2.7	3.0		1.0	8.3
Ni	0.456	9.5	5.0		1.0	21.5
Pb	0.102	5.6	14.9		1.0	31.8
Rb	2.46	1.5	4.0		1.2	8.7
Sb	0.010	13.6	5.9		0.3	29.8
Se	2.59	2.2	1.0	0.8	1.6	7.9
Sn	0.082	10.30	17.7		0.5	41.1
Sr	128	2.5	3.8	1.4	0.4	9.5
V	1.46	5.6	2.1	1.0	0.3	12.1
Zn	66.4	1.6	1.2		0.7	4.1

* mg kg^{-1} as Hg

TABLE 6. CERTIFIED VALUES FOR TRACE ELEMENT MASS FRACTIONS AND THEIR EXPANDED UNCERTAINTY (*k=2*) IN IAEA-407A

Element	Unit	Certified value[1]	Expanded uncertainty (*k*=2)[2]
As	mg kg^{-1}	13.33	0.62
Ca	mg kg^{-1}	24.5 × 10^3	3.0 × 10^3
Cd	mg kg^{-1}	0.184	0.021
Cu	mg kg^{-1}	3.09	0.21
Hg	mg kg^{-1}	0.219	0.011
K	mg kg^{-1}	12.83 × 10^3	0.95 × 10^3
MeHg	mg kg^{-1}as Hg	0.193	0.012
Mg	mg kg^{-1}	2.39 × 10^3	0.22 × 10^3
Mn	mg kg^{-1}	3.49	0.39
Na	mg kg^{-1}	14.2 ×10^3	1.2 × 10^3
Rb	mg kg^{-1}	2.46-	0.22
Se	mg kg^{-1}	2.6	0.2
Sr	mg kg^{-1}	128	12
V	mg kg^{-1}	1.46	0.18
Zn	mg kg^{-1}	66.4	2.7

[1] The value is the mean of the means of the accepted 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] The uncertainty is expressed as a combined standard uncertainty with a coverage factor *k*=2, corresponding to the level of confidence of about 95%, estimated in accordance with the JCGM 100:2008 Evaluation of measurement data – Guide to the expression of uncertainty in measurement [12], and ISO Guide 35 [6].

TABLE 7. INFORMATION VALUES FOR TRACE ELEMENT MASS FRACTIONS AND THEIR EXPANDED UNCERTAINTY (*k=2*) IN IAEA-407A

Element	Unit	Information value[1]	Expanded uncertainty (*k*=2)[2]
Ag	mg kg^{-1}	0.035	0.007
Al	mg kg^{-1}	11.1	4.6
Co	mg kg^{-1}	0.089	0.018
Cr	mg kg^{-1}	0.67	0.20
Li	mg kg^{-1}	0.697	0.130
Ni	mg kg^{-1}	0.456	0.098
Pb	mg kg^{-1}	0.102	0.033
Sb	mg kg^{-1}	0.010	0.003
Sn	mg kg^{-1}	0.082	0.034

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

[2] The uncertainty is expressed as a combined standard uncertainty with a coverage factor *k*=2, corresponding to the level of confidence of about 95%, estimated in accordance with the JCGM 100:2008 Evaluation of measurement data – Guide to the expression of uncertainty in measurement [12], and ISO Guide 35 [6].

4. METROLOGICAL TRACEABILITY AND COMMUTABILITY

If the results obtained by different laboratories are to be compared, it is essential that all results are based on reliable measurement standards, the values of which are linked to a common reference.

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 (National Institute of Standards and Technology (NIST), Bundesanstalt für Materialforschung und -prüfung (BAM) or Swiss Federal Laboratories for Materials Science and Technology (EMPA)) with demonstrated SI traceability, followed by gravimetric preparation using balances calibrated with SI-traceable weights.

Only validated methods, applied within the stated scope of validation, were used by participating laboratories in this characterization study. Matrix CRMs with stated SI traceability purchased from NIST, European Commission, Joint Research Centre (JRC), Directorate F - Health, Consumers and Reference Materials (EC JRC IRMM), National Research Council of Canada (NRC) and IAEA were used for validation of the methods applied in this characterization exercise.

As the certified values are combinations of agreeing results, individually traceable to the SI system of units, the certified quantity values are also traceable to the SI system of units. 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 [13].

Commutability is a critical requirement to avoid introduction of unintended, and sometimes undetected, bias in measurement results, when using a CRM. Commutable CRMs should exhibit an analytical behavior for a given method similar to the real laboratory sample. IAEA-407A is a homogenized fish sample, whose analytical behavior should be the same as the behavior of a routine fish sample. The agreement between results obtained with different analytical methods selected for the IAEA-407A characterization study confirms the absence of any significant method bias and demonstrates commutability of all certified trace elements mass fractions in the IAEA-407A biota sample.

5. CONCLUSIONS

This certification campaign allows assignment of certified values for As, Ca, Cd, Cu, Hg, K, MeHg, Mg, Mn, Na, Rb, Se, Sr, V and Zn with associated uncertainties following ISO guidelines. The certified values are derived from measurement results provided by the laboratories with demonstrated measurement performances, participating in the characterization study. Only validated methods were applied in the characterization of IAEA-407A CRM. As the certified values are derived from SI traceable individual results, they are also traceable to the SI International System of Units. The new CRM IAEA-407A can be successfully applied for quality assurance and procedure validations used in environmental monitoring and seafood safety studies.

APPENDIX I

RESULTS FROM THE LONG-TERM STABILITY STUDY

Figures 2–25 present individual mass fractions per time point. In all figures, the results are plotted versus storage duration. In addition, the certified (or informative) value is plotted as a bold line, while the dashed lines represent the certified (or informative) value ± its associated expanded uncertainty (*k*=2).

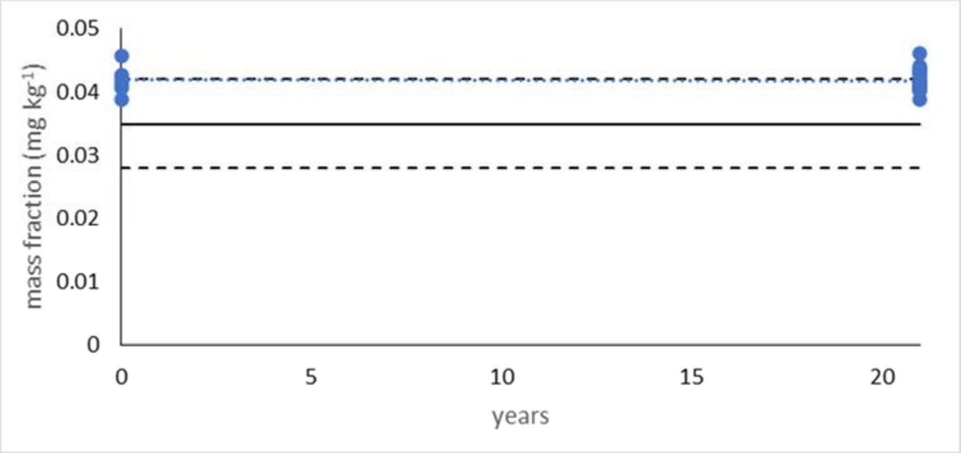

FIG. 2. Results of long-term stability study for silver.

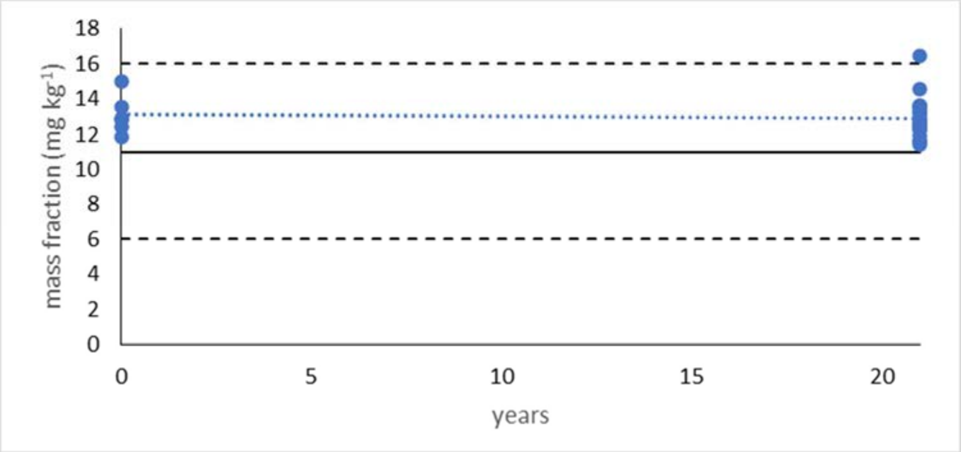

FIG. 3. Results of long-term stability study for aluminium.

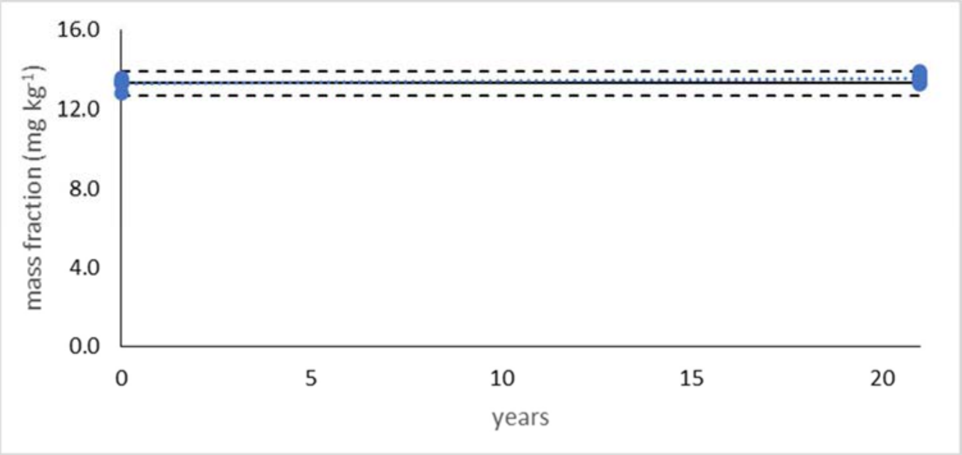

FIG. 4. Results of long-term stability study for arsenic.

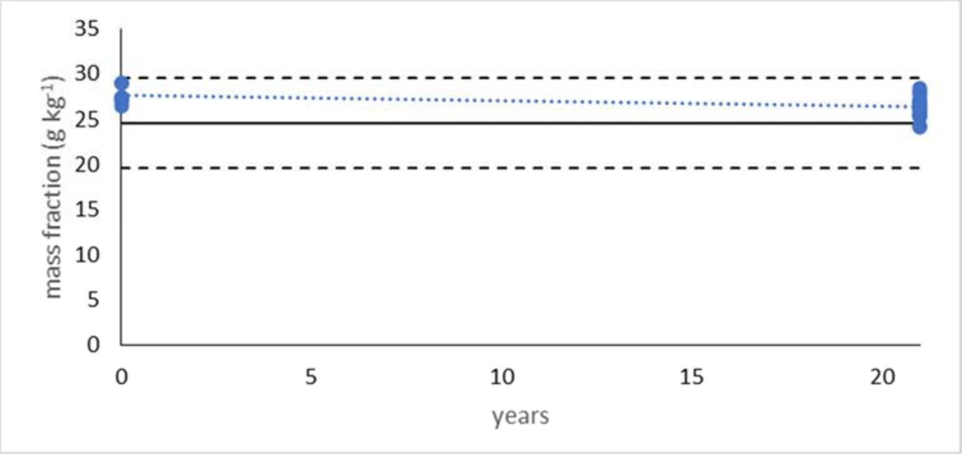

FIG. 5. Results of long-term stability study for calcium.

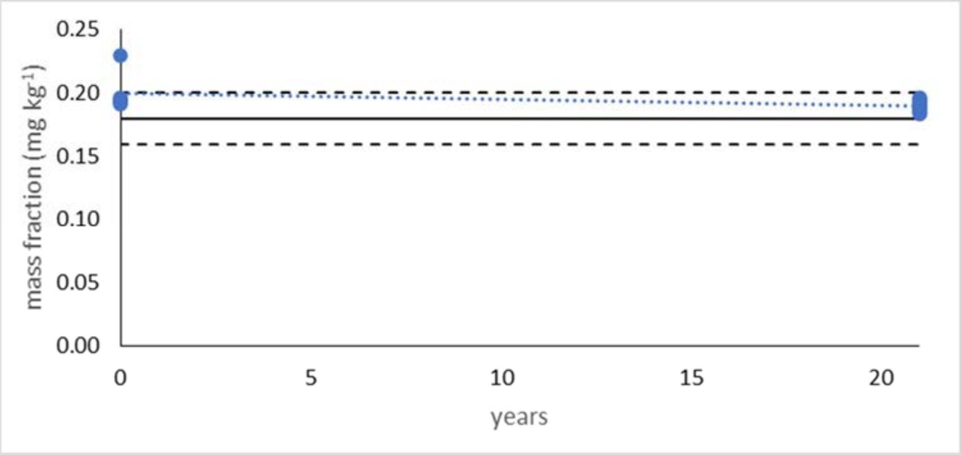

FIG. 6. Results of long-term stability study for cadmium.

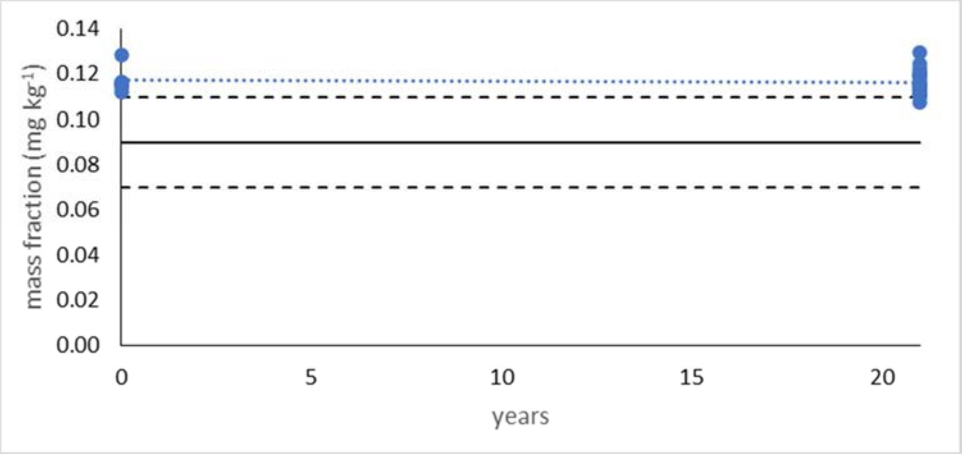

FIG. 7. *Results of long-term stability study for cobalt.*

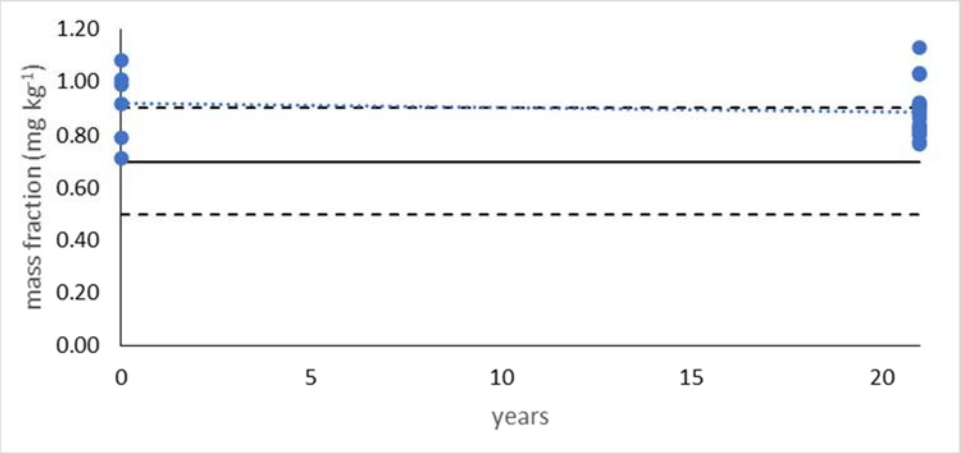

FIG. 8. *Results of long-term stability study for chromium.*

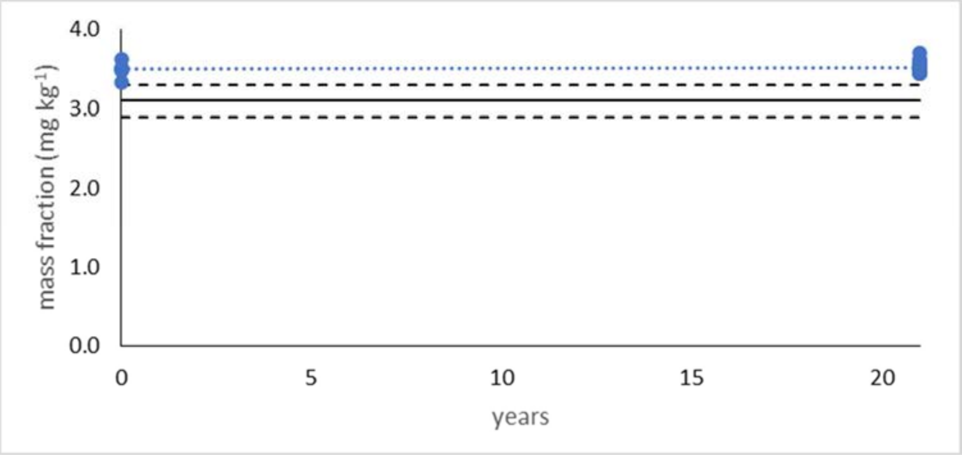

FIG. 9. *Results of long-term stability study for copper.*

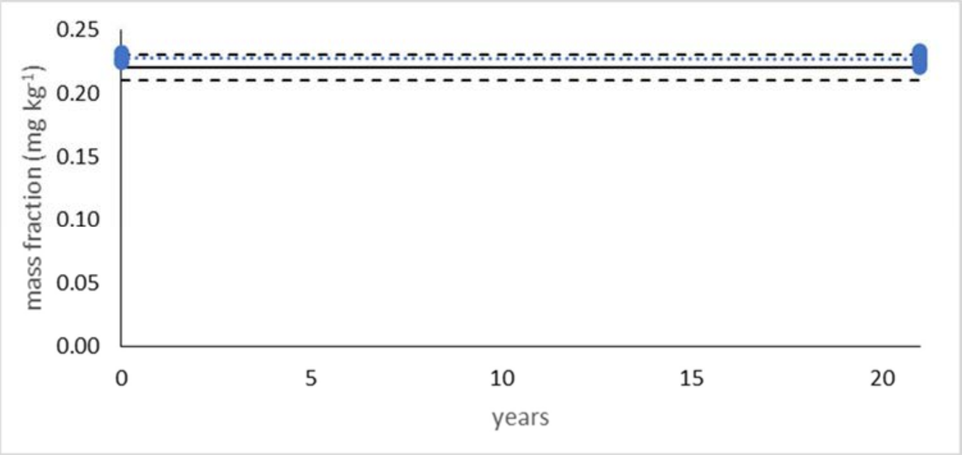

FIG. 10. *Results of long-term stability study for mercury.*

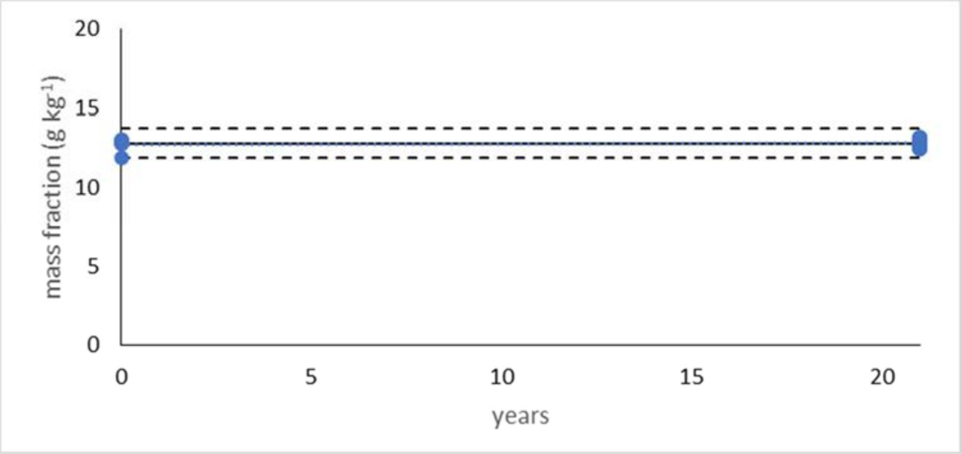

FIG. 11. *Results of long-term stability study for potassium.*

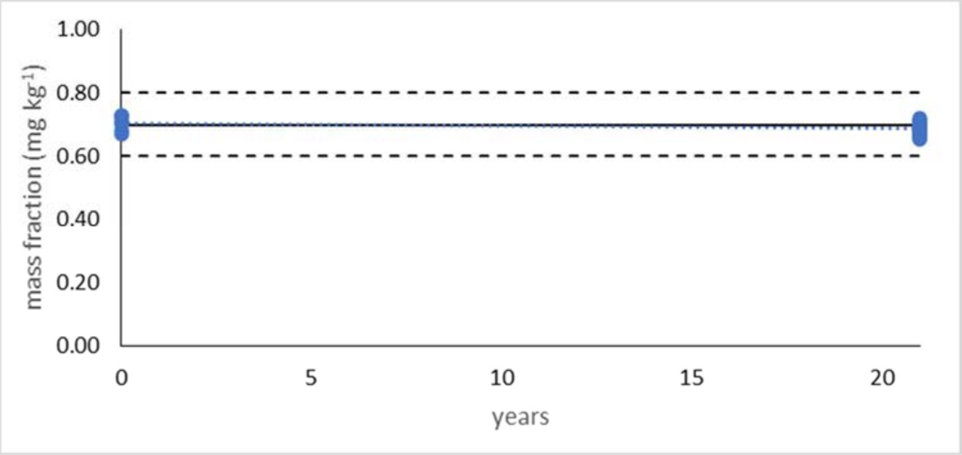

FIG. 12. *Results of long-term stability study for lithium.*

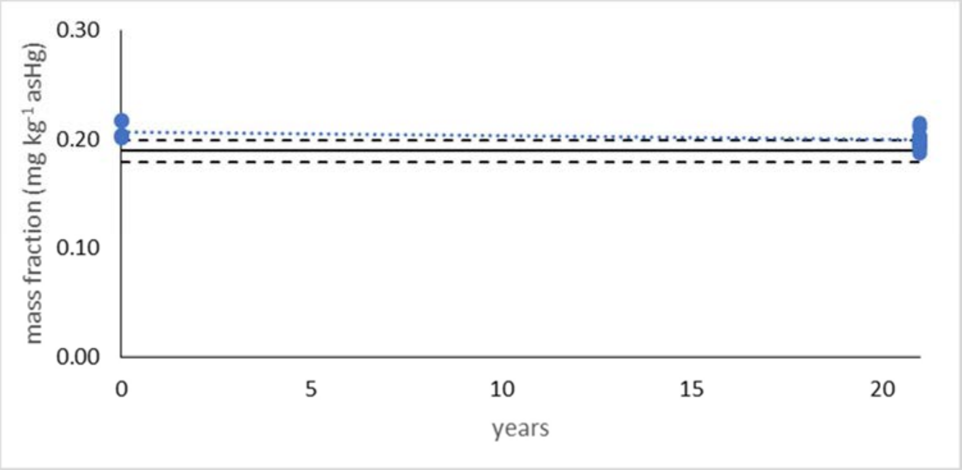

FIG. 13. Results of long-term stability study for methylmercury.

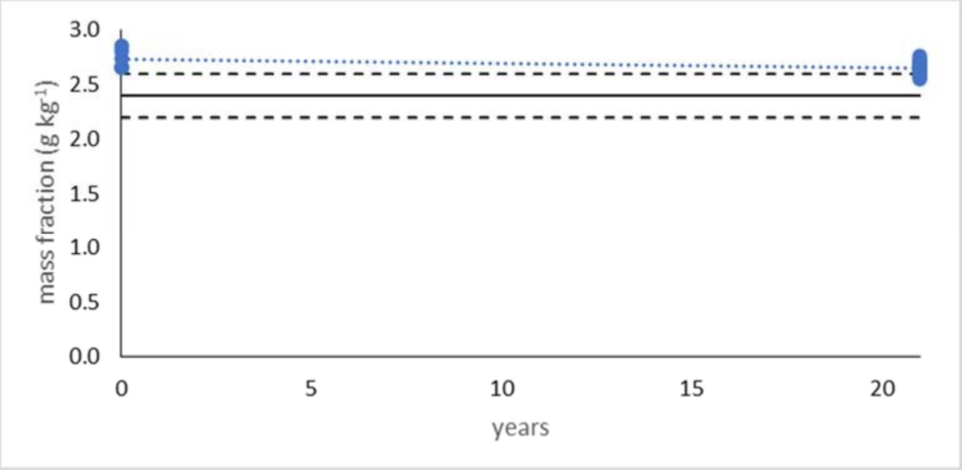

FIG. 14. Results of long-term stability study for magnesium.

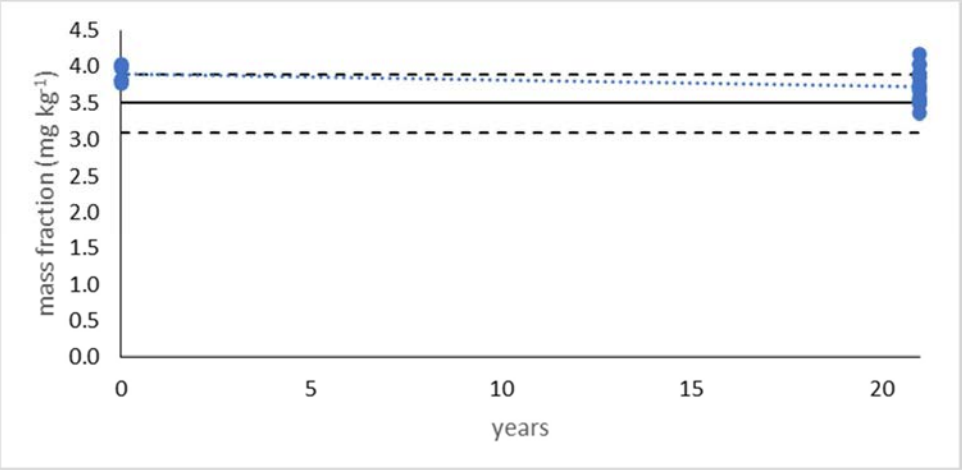

FIG. 15. Results of long-term stability study for manganese.

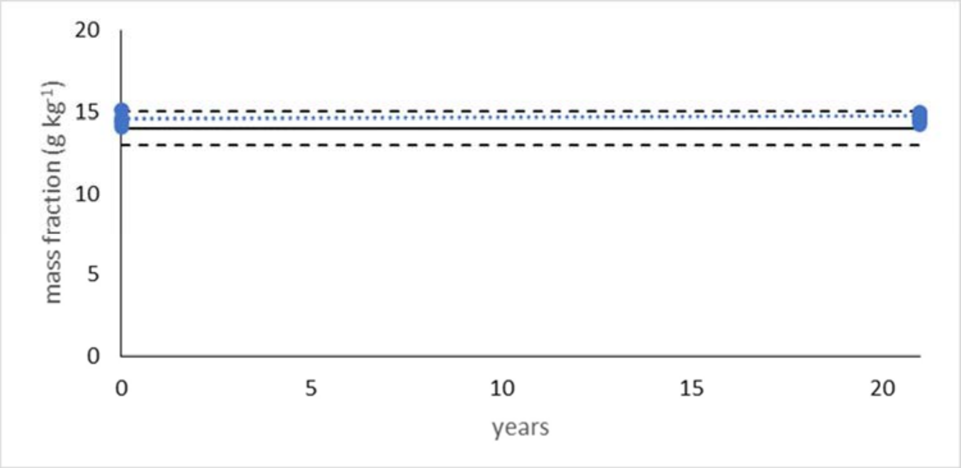

FIG. 16. Results of long-term stability study for sodium.

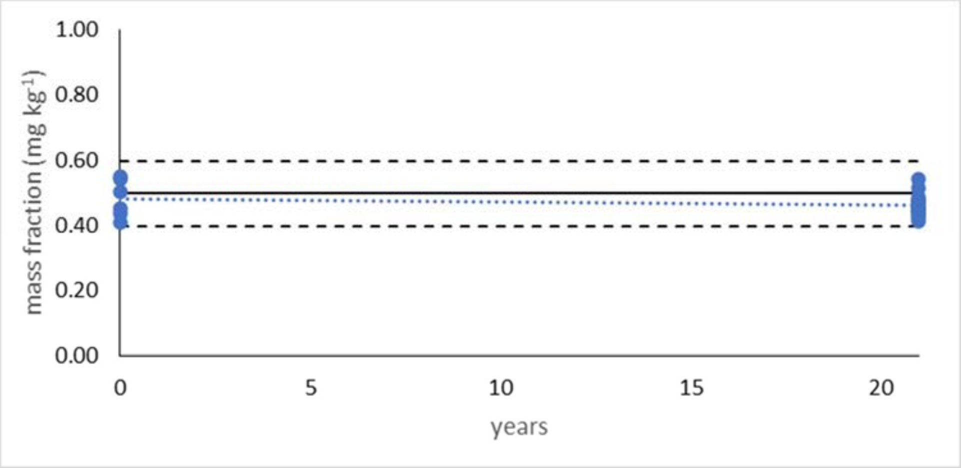

FIG. 17. Results of long-term stability study for nickel.

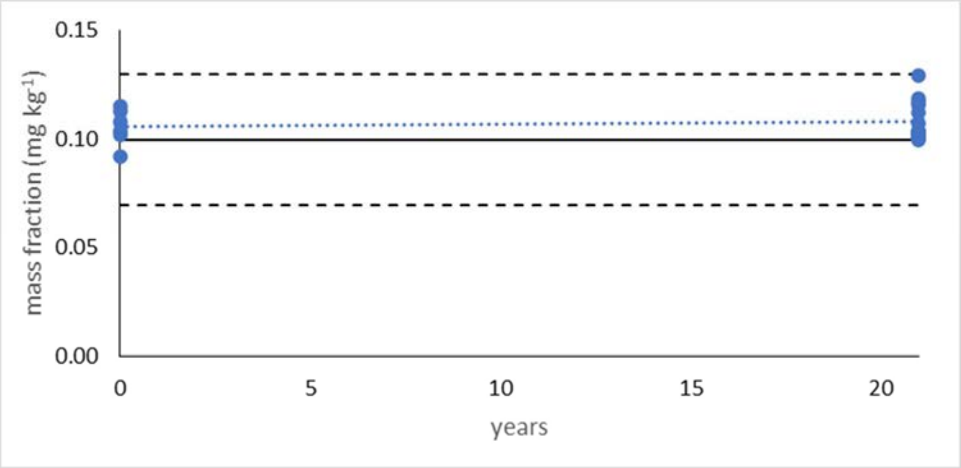

FIG. 18. Results of long-term stability study for lead.

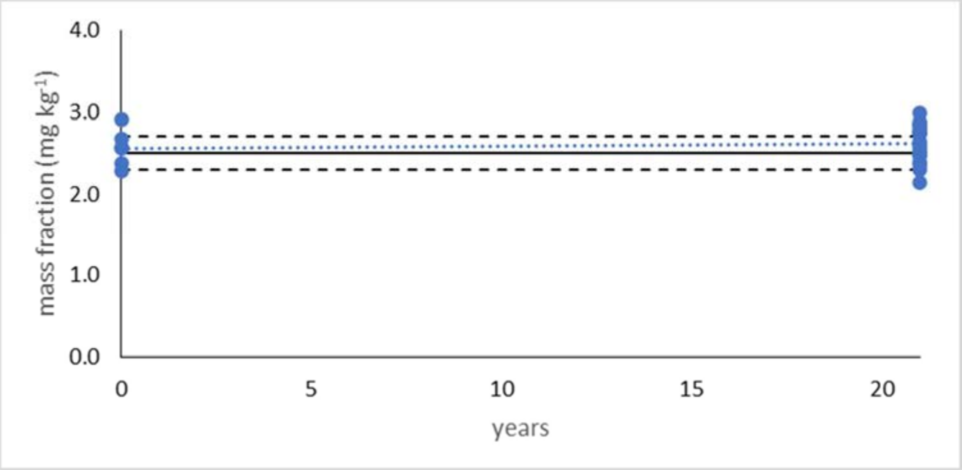

FIG. 19. Results of long-term stability study for rubidium.

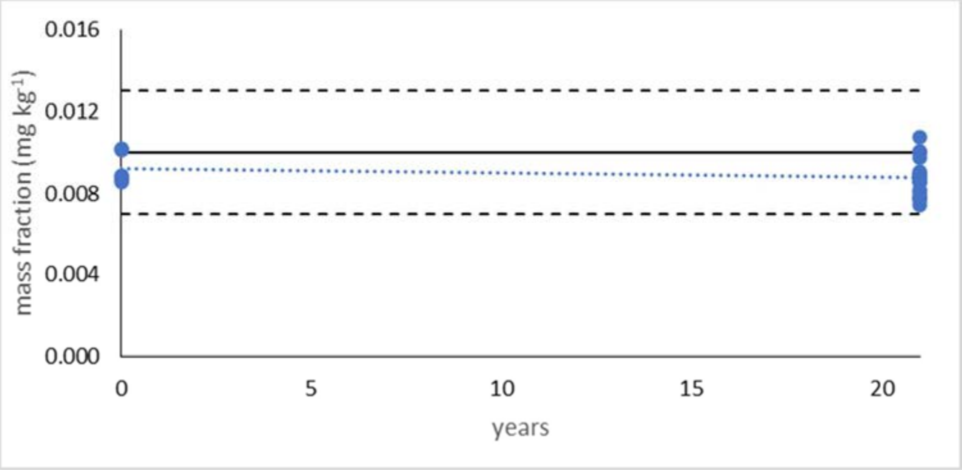

FIG. 20. Results of long-term stability study for antimony.

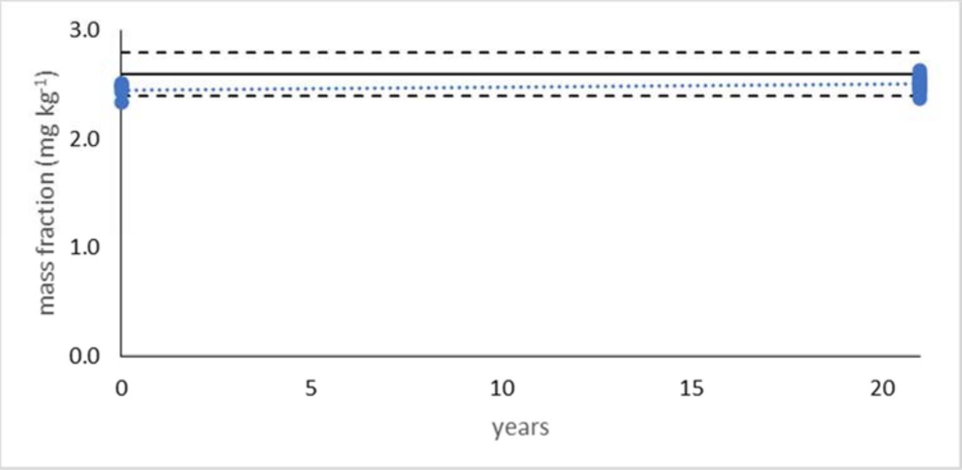

FIG. 21. Results of long-term stability study for selenium.

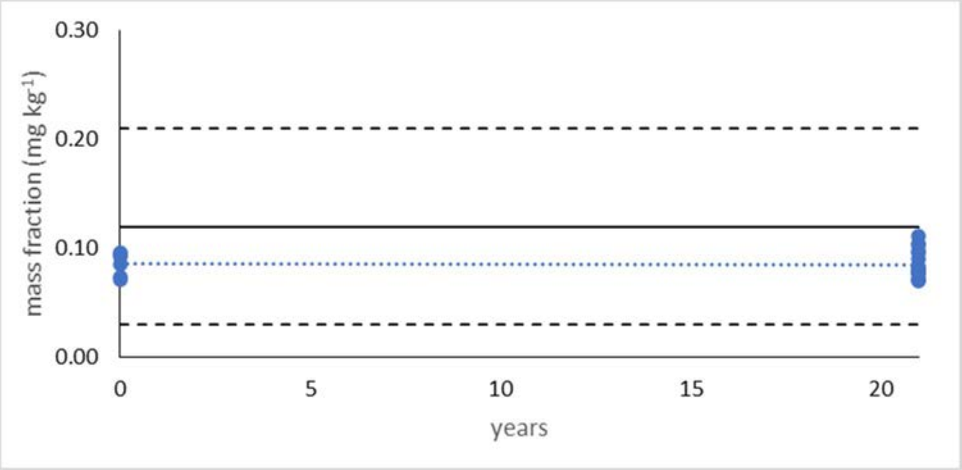

FIG. 22. Results of long-term stability study for tin.

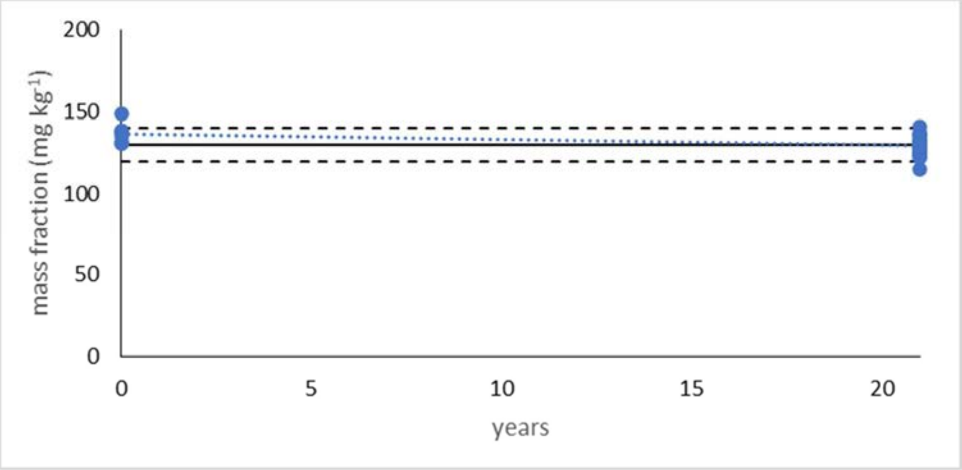

FIG. 23. Results of long-term stability study for strontium.

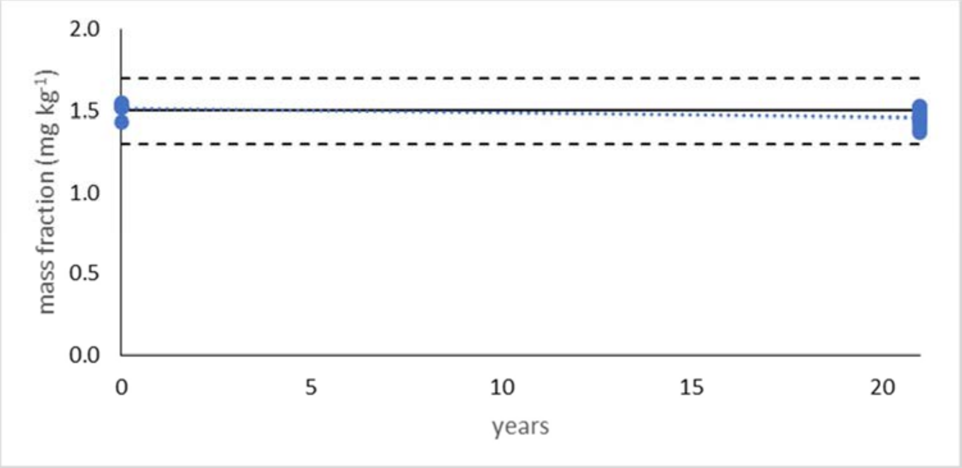

FIG. 24. Results of long-term stability study for vanadium.

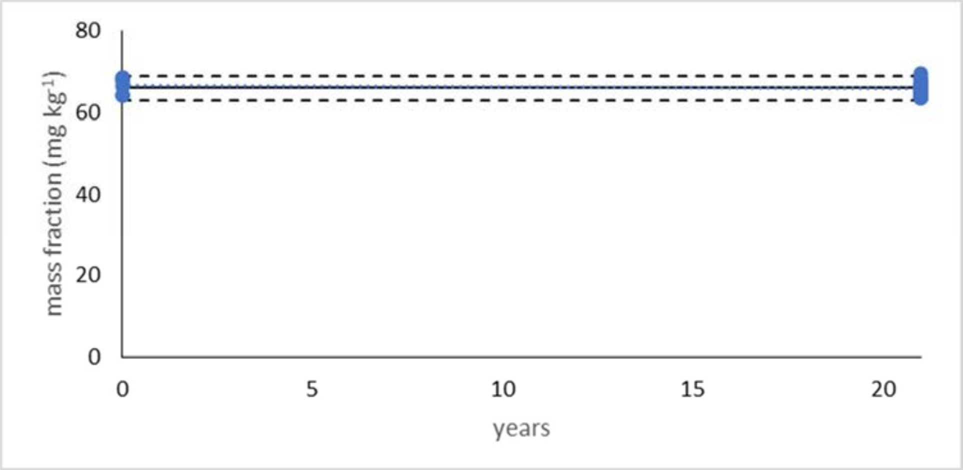

FIG. 25. Results of long-term stability study for zinc.

APPENDIX II

RESULTS FROM THE SHORT-TERM STABILITY STUDY

Figures 26–48 present individual mass fractions per time point. In all figures the results are plotted versus storage duration. In addition, the certified (or informative) value is plotted as a bold line, while the dashed lines represent the certified (or informative) value ± its expanded uncertainty (k=2) associated.

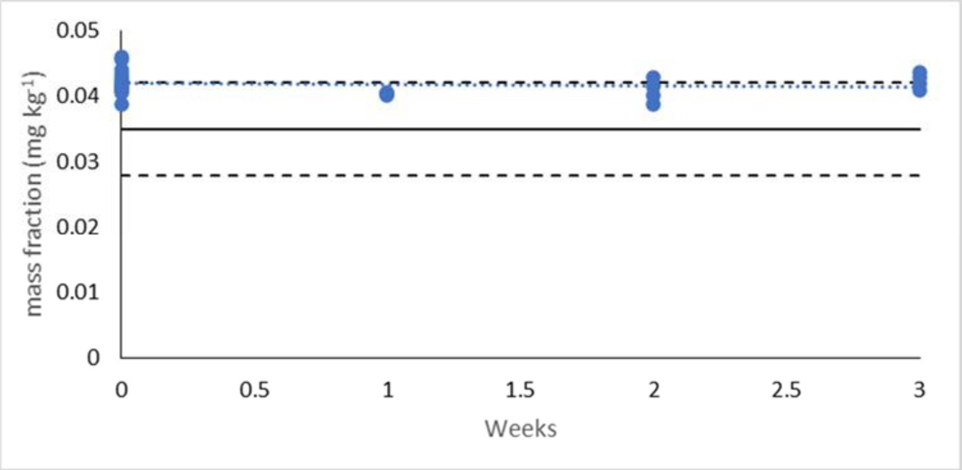

FIG. 26. Results of short-term stability study for silver.

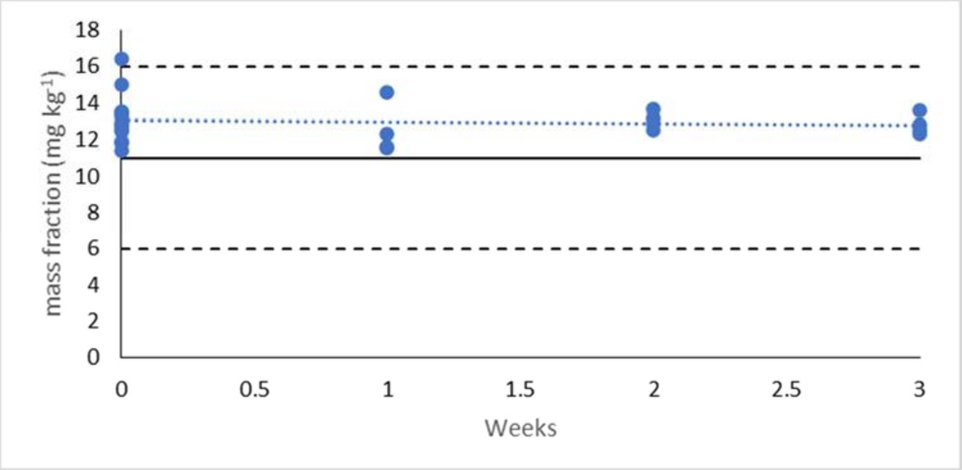

FIG. 27. Results of short-term stability study for aluminium.

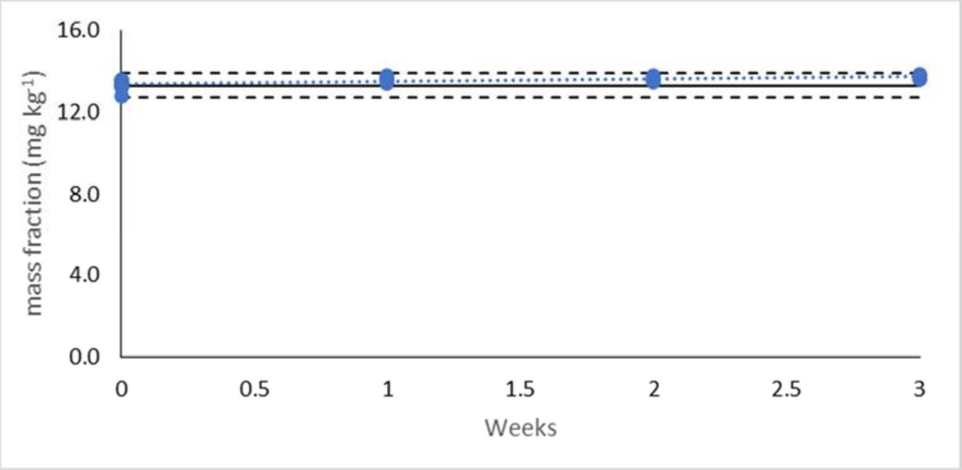

FIG. 28. Results of short-term stability study for arsenic.

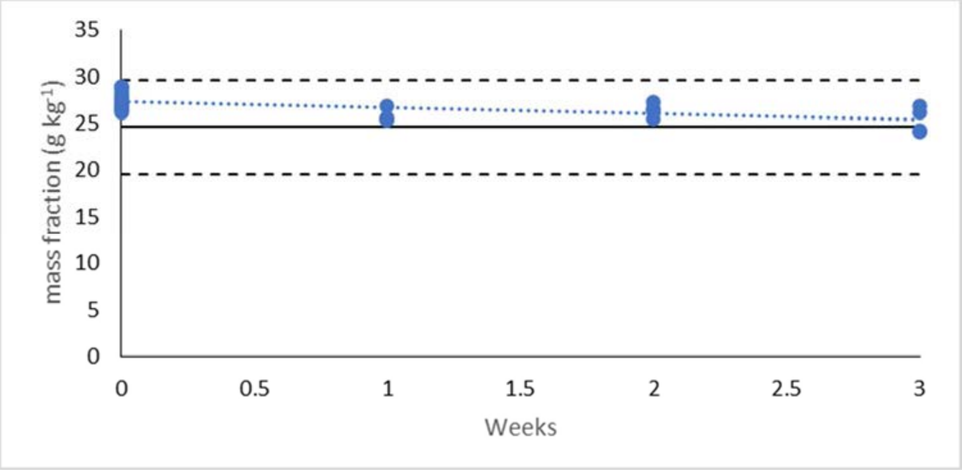

FIG. 29. Results of short-term stability study for calcium.

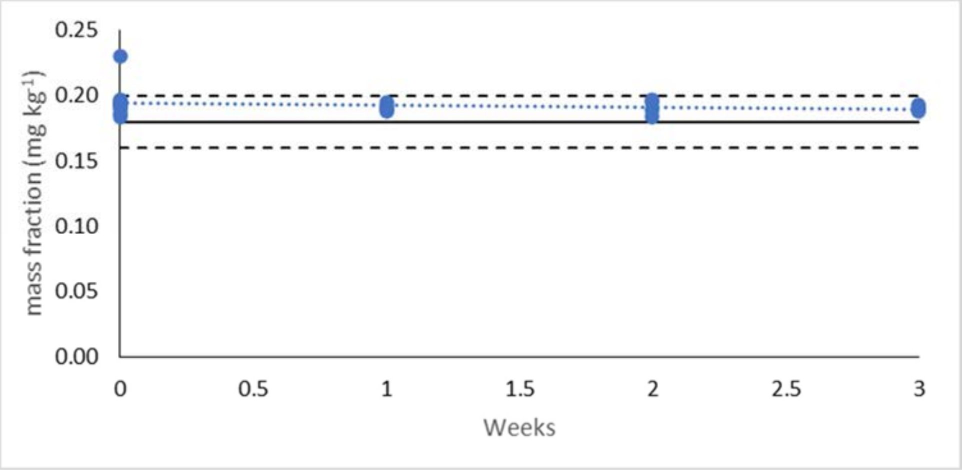

FIG. 30. Results of short-term stability study for cadmium.

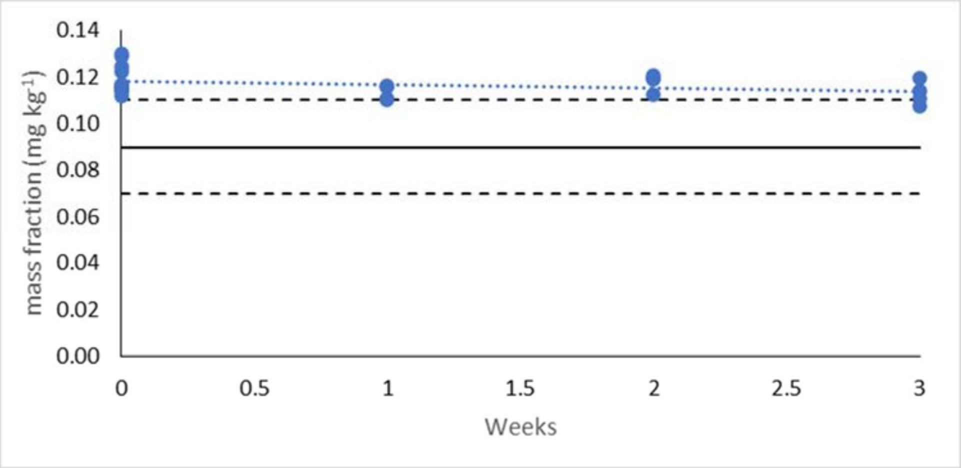

FIG. 31. *Results of short-term stability study for cobalt.*

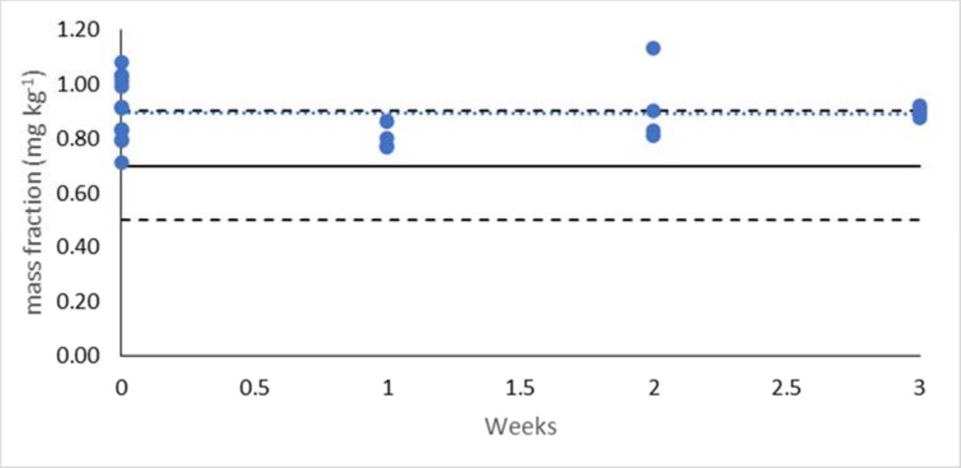

FIG. 32. *Results of short-term stability study for chromium.*

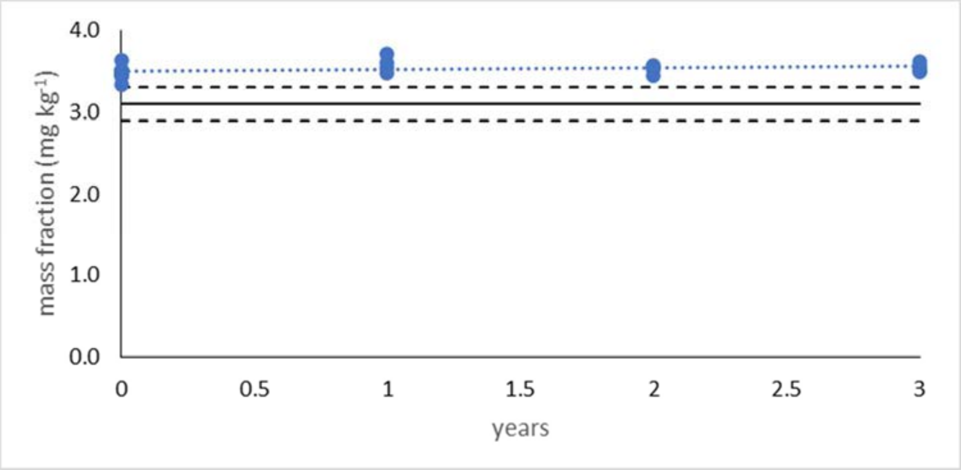

FIG. 33. *Results of short-term stability study for copper.*

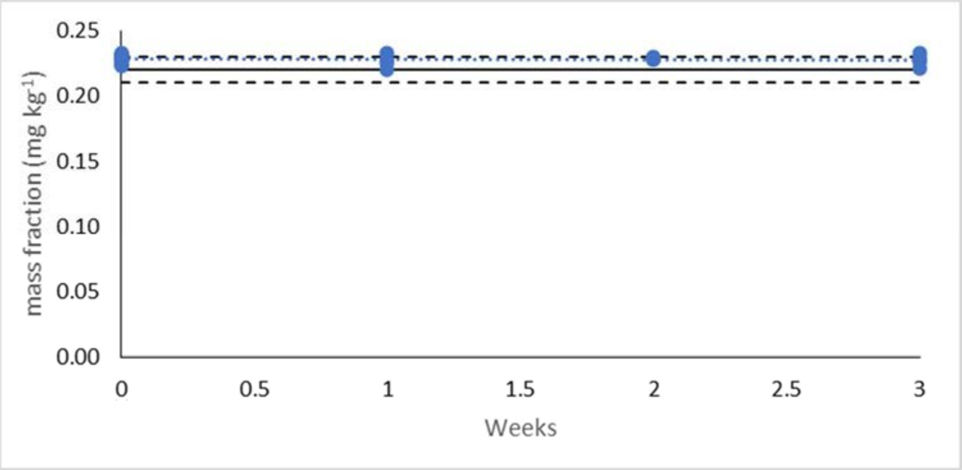

FIG. 34. Results of short-term stability study for mercury.

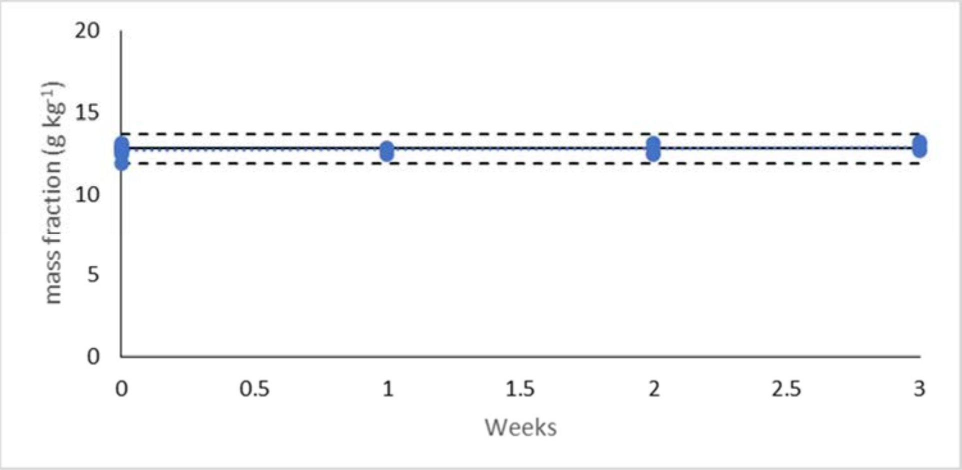

FIG. 35. Results of short-term stability study for potassium.

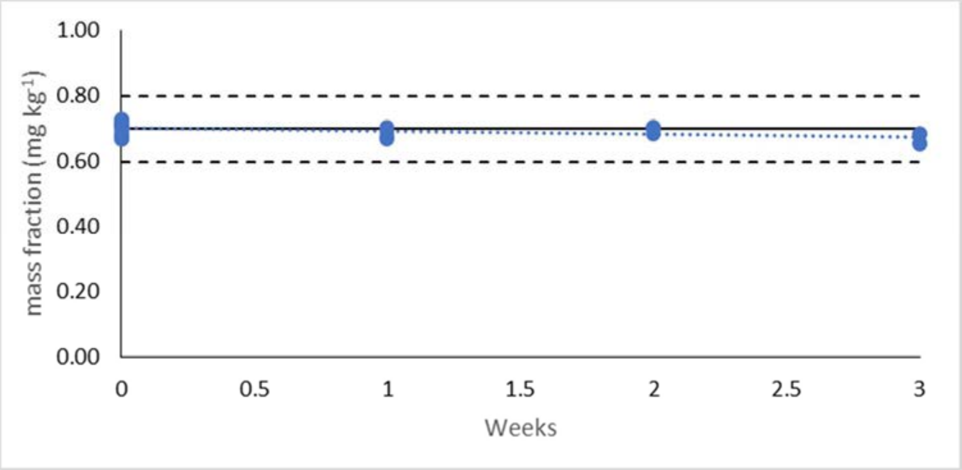

FIG. 36. Results of short-term stability study for lithium.

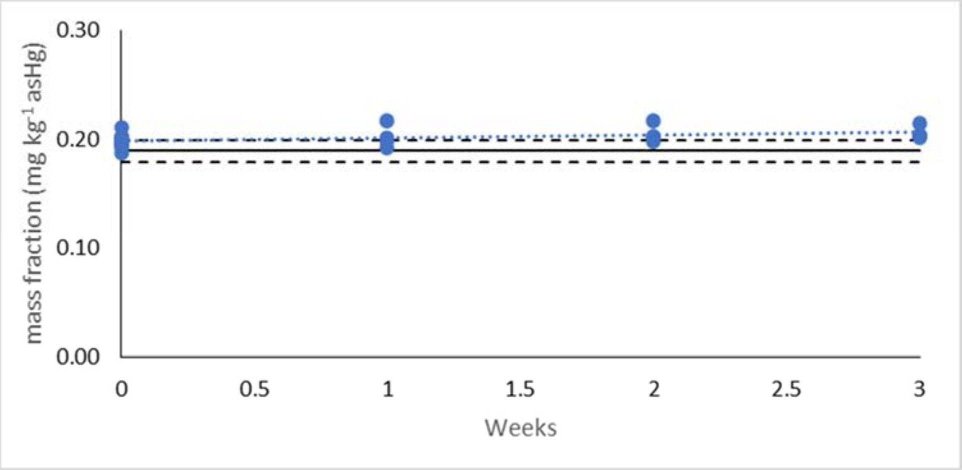

FIG. 37. Results of short-term stability study for methylmercury.

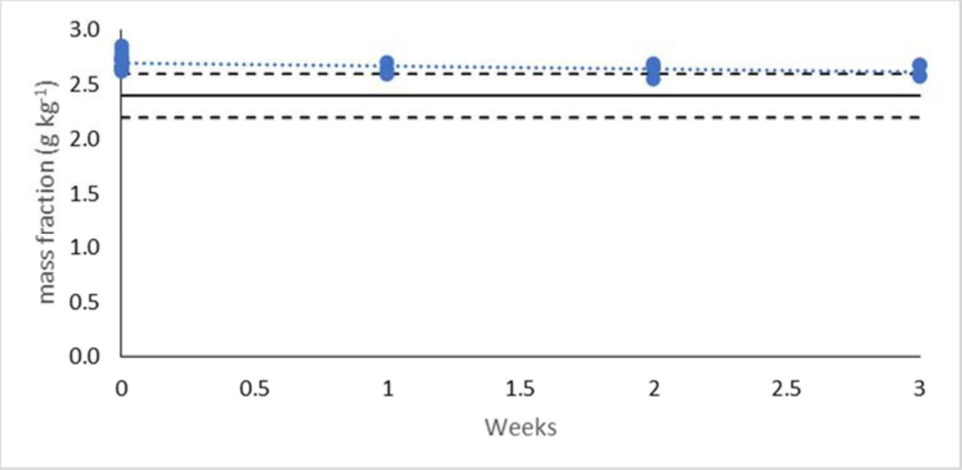

FIG. 37. Results of short-term stability study for magnesium.

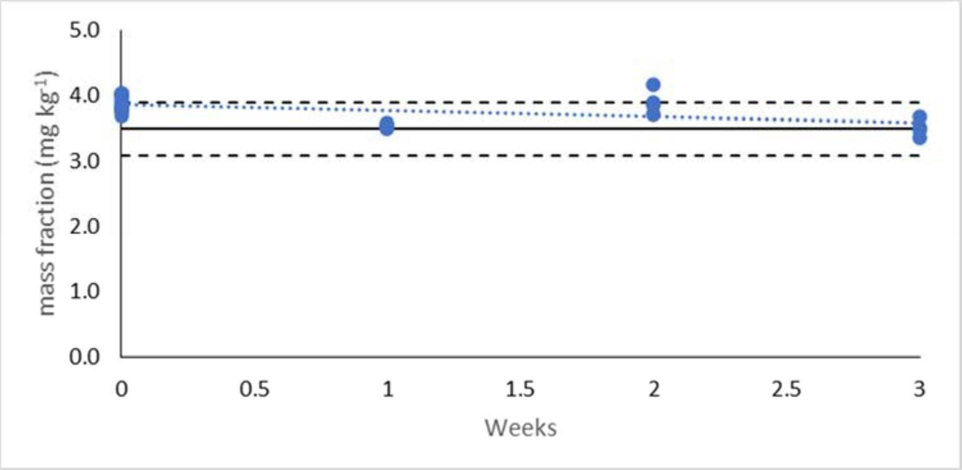

FIG. 38. Results of short-term stability study for manganese.

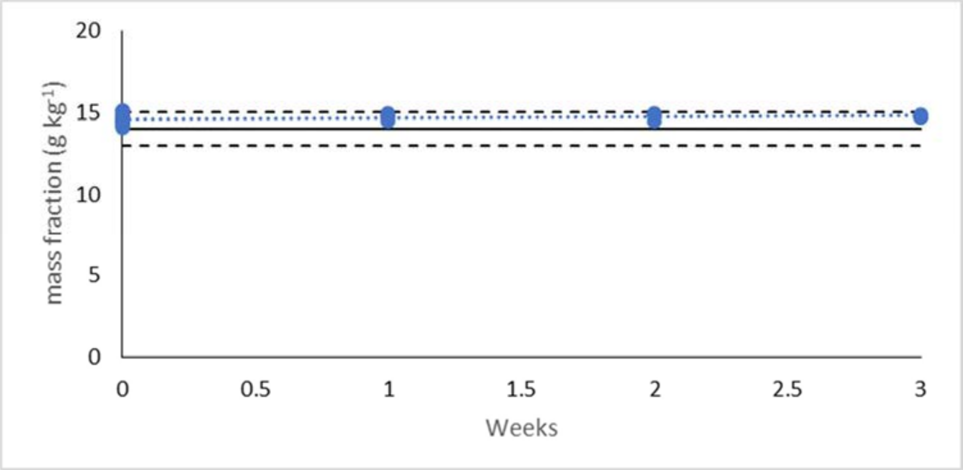

FIG. 39. Results of short-term stability study for sodium.

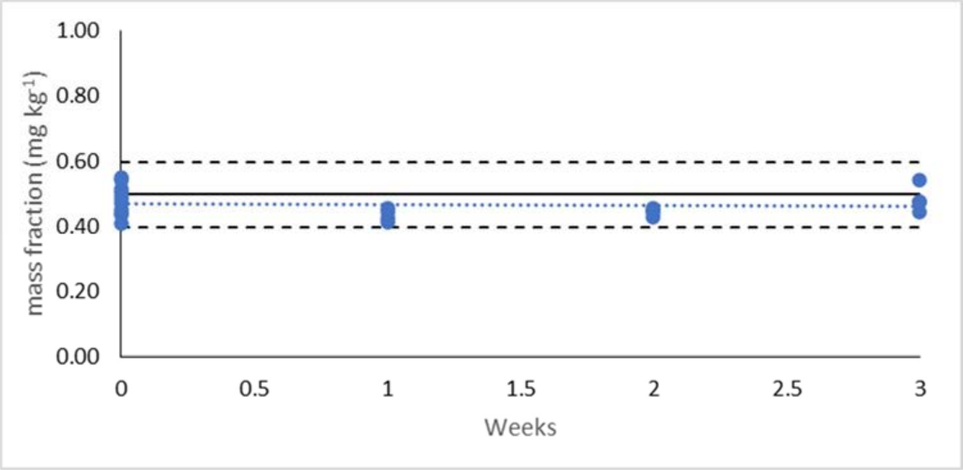

FIG. 40. Results of short-term stability study for nickel.

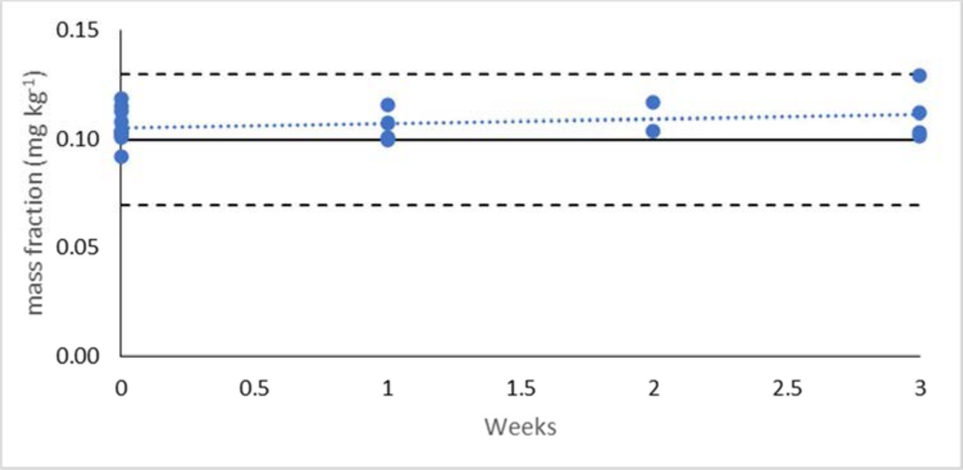

FIG. 41. Results of short-term stability study for lead.

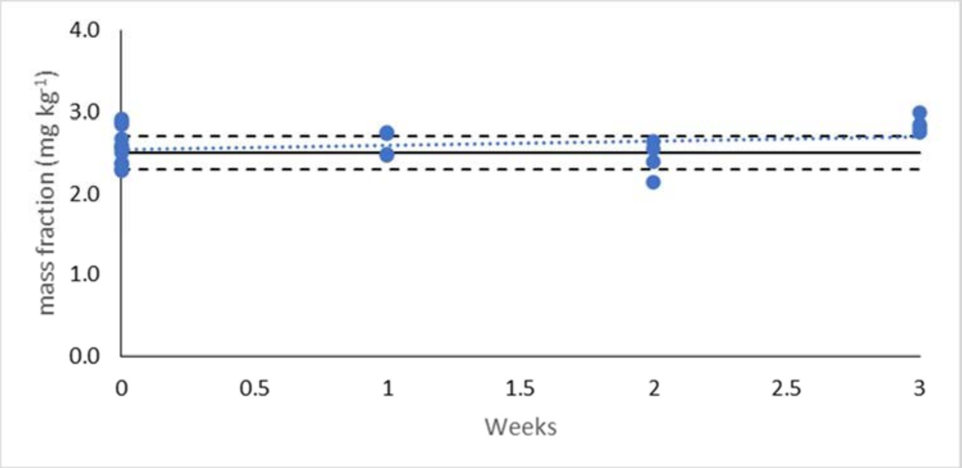

FIG. 42. Results of short-term stability study for rubidium.

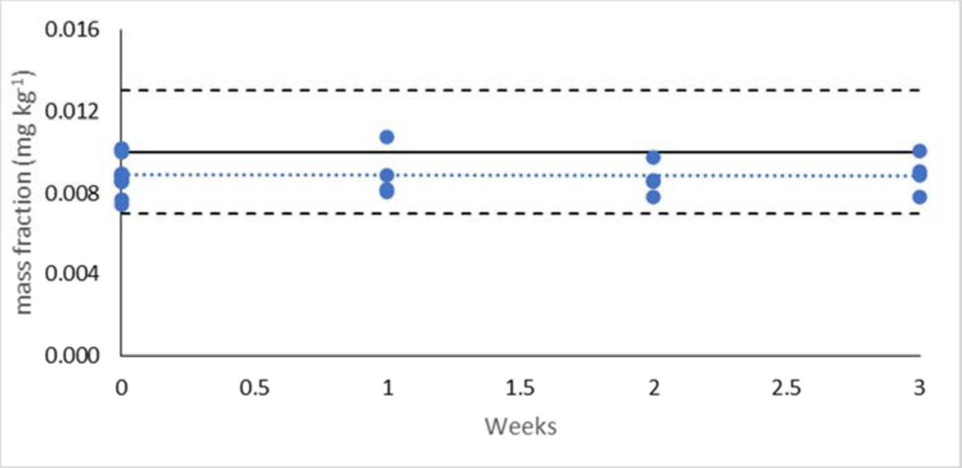

FIG. 43. Results of short-term stability study for antimony.

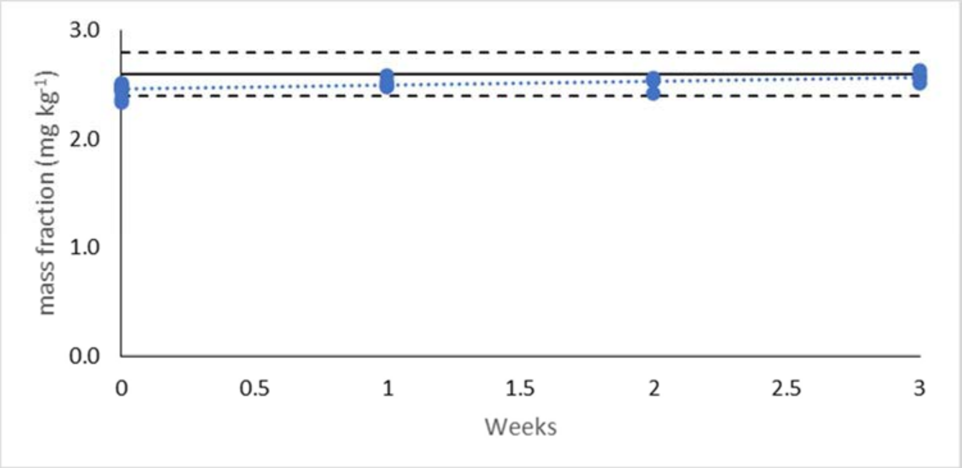

FIG. 44. Results of short-term stability study for selenium.

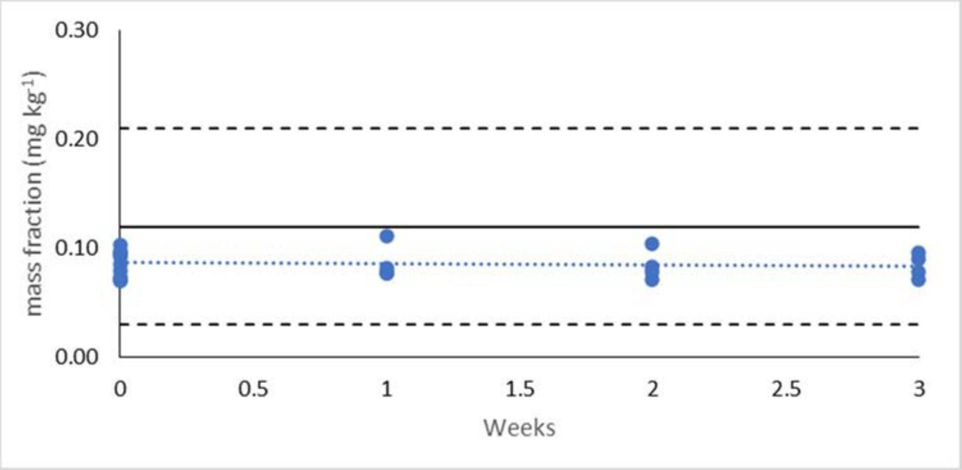

FIG. 45. Results of short-term stability study for tin.

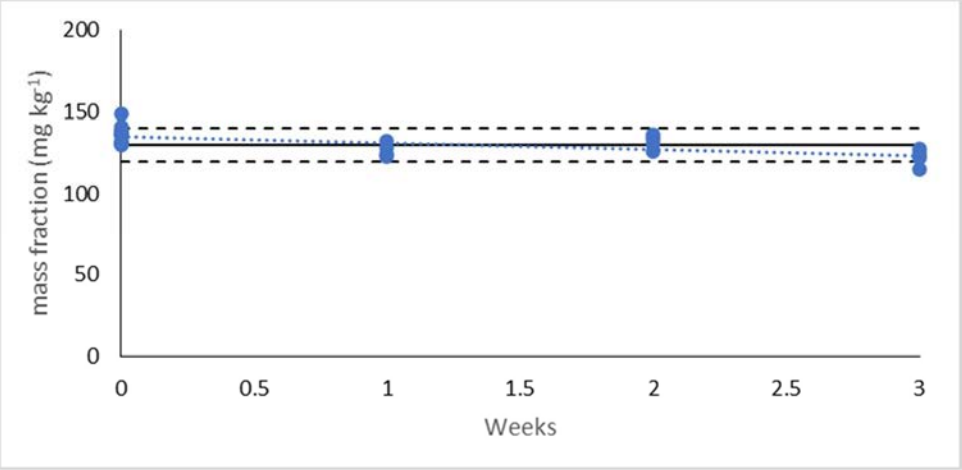

FIG. 46. Results of short-term stability study for strontium.

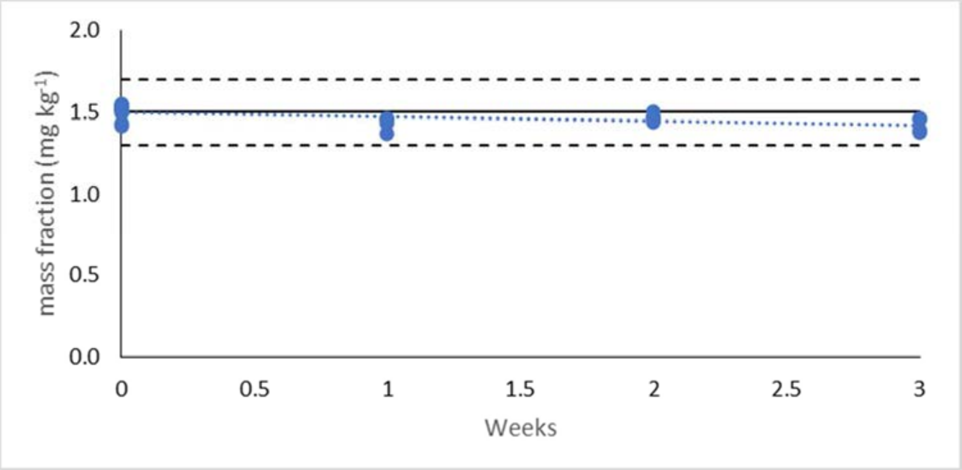

FIG. 47. Results of short-term stability study for vanadium.

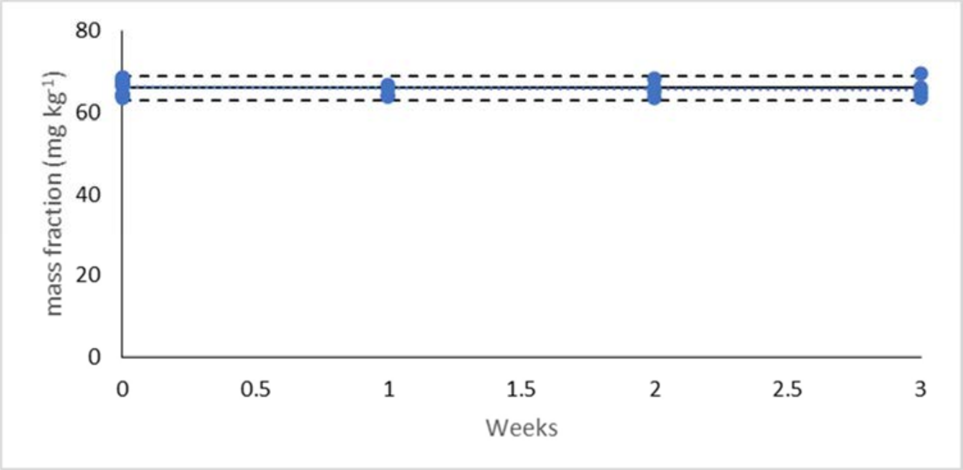

FIG. 48. Results of short-term stability study for zinc.

APPENDIX III

RESULTS OF THE CHARACTERIZATION MEASUREMENTS

The results reported by the participating laboratories, their expanded uncertainty, measurement techniques and CRMs used for quality assurance purposes are presented in Tables 8-22, results not considered after technical evaluation (2.2) are shaded in grey. Figures 49 – 63 provide graphical presentation of the individual results and their expanded uncertainties (*k*=2) as well as the reference value for the respective trace element and its expanded uncertainty (*k*=2).

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

Laboratory code	Mean	Expanded uncertainty (*U*)	CRM	Method
1	12.8	1.0	DORM-4	Neutron Activation
2	13.9	0.7	IAEA 476	ICP-MS
4	10.3	1.2	DORM-4	ICP-OES
6	9.8	3.9	Oyster 1566b	Graphite Furnace AAS
7	11.6	0.4	DORM-4	ICP-MS
9	13.6	1.5	DORM-4	ICP-MS
10	15.7	1.0	CE 278K	ICP-MS
12	12.1	1.3	IAEA 407	Neutron Activation
13	13.9	2.1	DORM-2	ICP-MS
IAEA 1	13.9	2.4	IAEA 476	Graphite Furnace AAS
IAEA 2	13.2	2.0	IAEA 476	ICP-MS

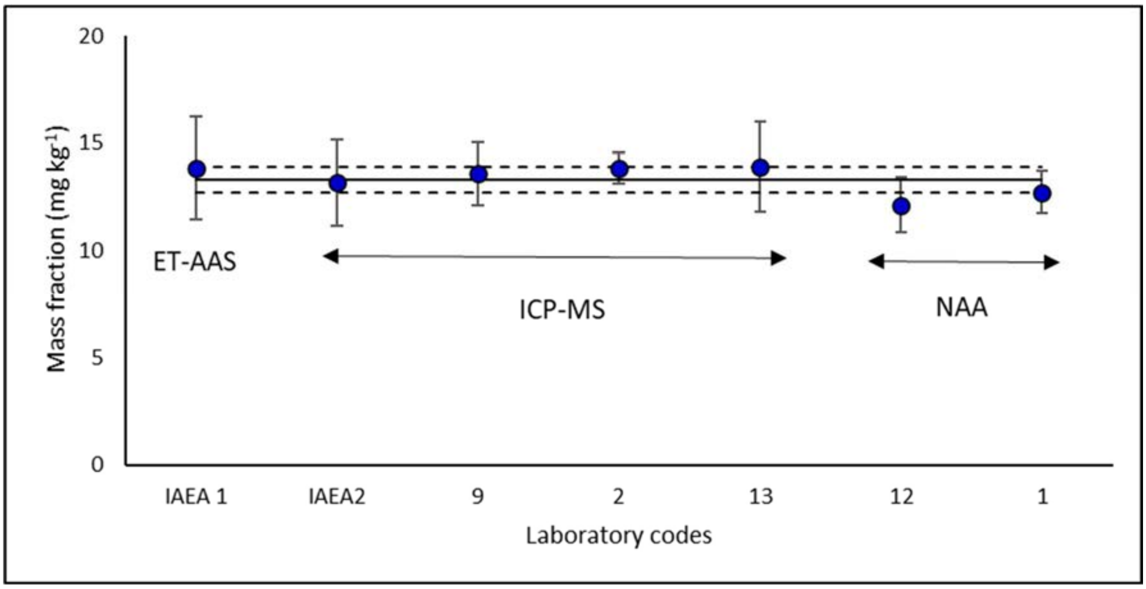

FIG. 49. Laboratory results for arsenic mass fraction (mg kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	23.3	1.6	DORM-4	Neutron Activation
4	24.8	1.2	DORM-4	ICP-OES
7	22.6	1.3	DORM-4	ICP-MS
9	27.2	1.4	DORM-4	ICP-MS
12	19.7	0.9	GRASS GR-96 (WEPAL)	XRF
13	24.2	3.6	DORM-4	ICP-MS
IAEA 1	28.2	3.4	IAEA 407	Flame AAS

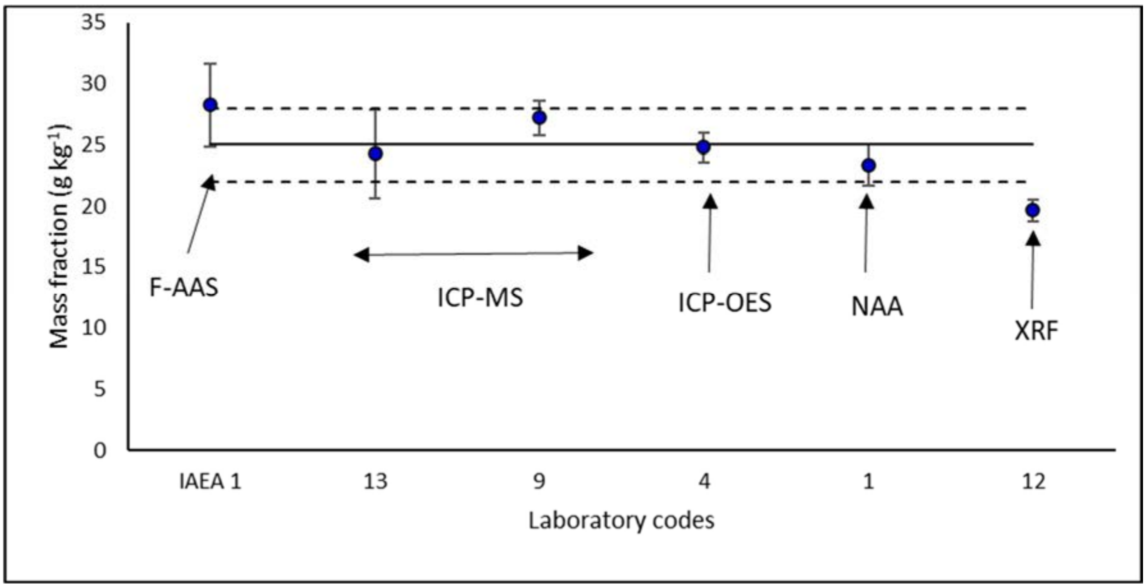

FIG. 50. Laboratory results for calcium mass fraction (g kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	0.193	0.016	IAEA 436A	ICP-MS
4	0.154	0.006	DORM-4	ICP-MS
6	0.187	0.037	DORM-4	Graphite Furnace AAS
7	0.170	0.009	DORM-4	ICP-MS
9	0.170	0.022	DORM-4	ICP-MS
10	0.179	0.012	CE 278K	ICP-MS
13	0.184	0.028	DORM-2	ICP-MS
IAEA-1	0.191	0.050	IAEA 476	Graphite Furnace AAS
IAEA-2	0.191	0.027	IAEA 476	ICP-MS
IAEA-4	0.188	0.005		Isotope Dilution ICP-MS

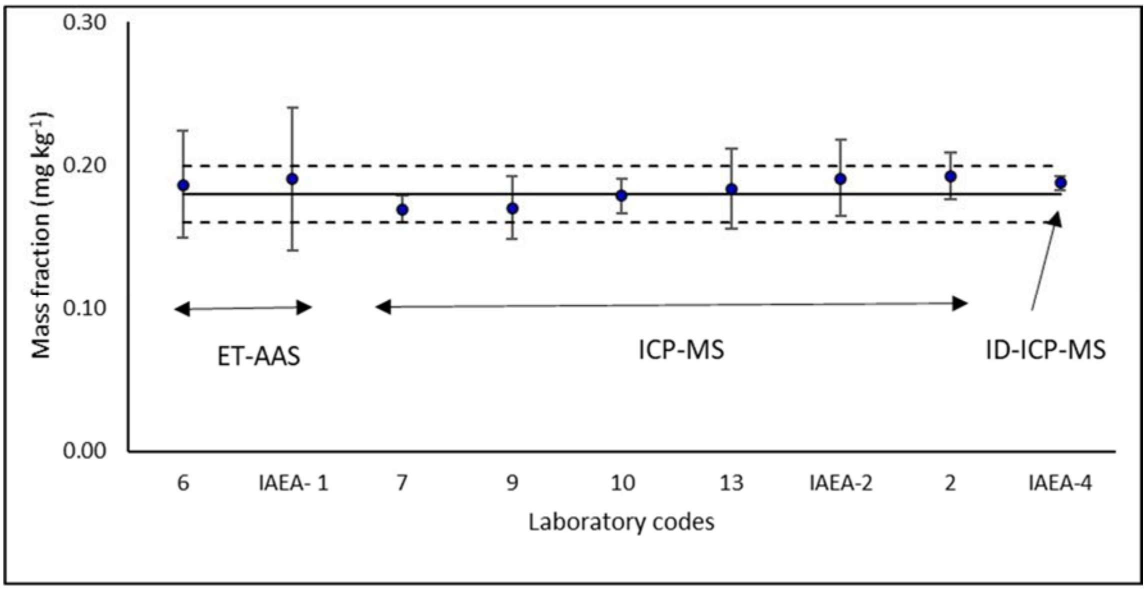

FIG. 51. Laboratory results for cadmium mass fraction (mg kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	3.47	0.18	IAEA 476	ICP-MS
4	2.40	0.46	DORM-4	ICP-MS
6	2.88	0.46	DORM-4	Flame AAS
7	2.77	0.17	DORM-4	ICP-MS
9	2.94	0.30	DORM-4	ICP-MS
10	2.93	0.20	CE 278K	ICP-MS
13	2.97	0.45	DORM-4	ICP-MS
IAEA-1	2.98	0.46	IAEA 476	Graphite Furnace AAS
IAEA-2	3.64	0.07	IAEA 476	ICP-MS
IAEA-4	3.22	0.09		Isotope Dilution ICP-MS

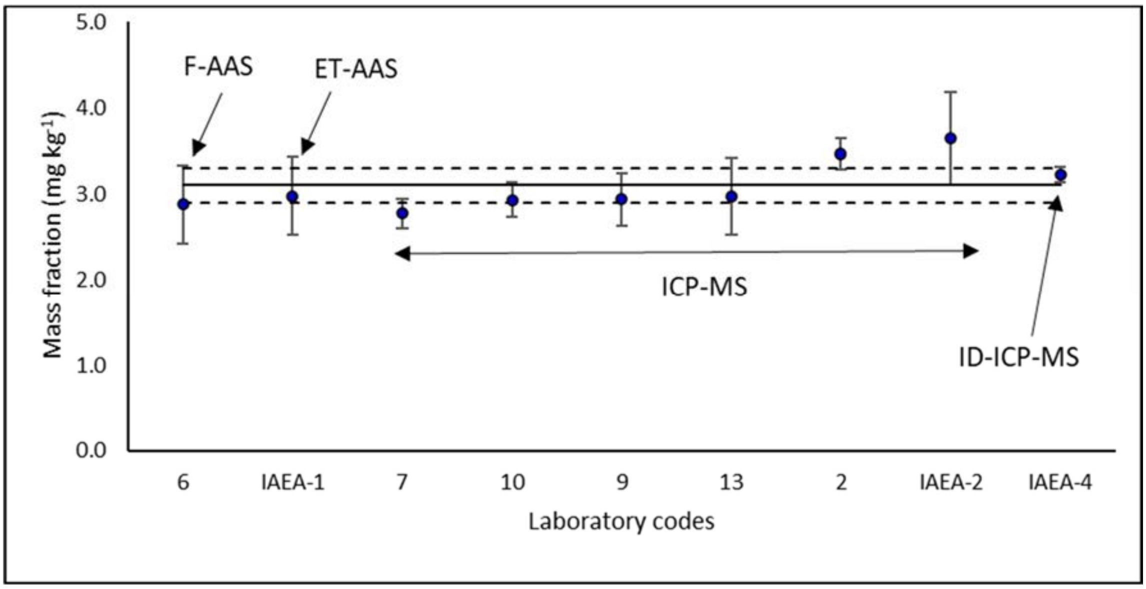

FIG. 52. Laboratory results for copper mass fraction (mg kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	0.241	0.020	DORM-4	Cold Vapor AAS
2	0.216	0.026	IAEA 476	ICP-MS
3	0.214	0.036	DORM-4	Solid AAS
6	0.219	0.048	DORM-4	Cold Vapor AFS
7	0.253	0.003	TORT-3	Cold Vapor AFS
9	0.195	0.029	DORM-4	Cold Vapor AFS
10	0.209	0.010	IAEA 407	Solid AAS
13	0.226	0.034	DORM-4	Cold Vapor ICP-MS
IAEA-1	0.233	0.028	IAEA 476	Solid AAS

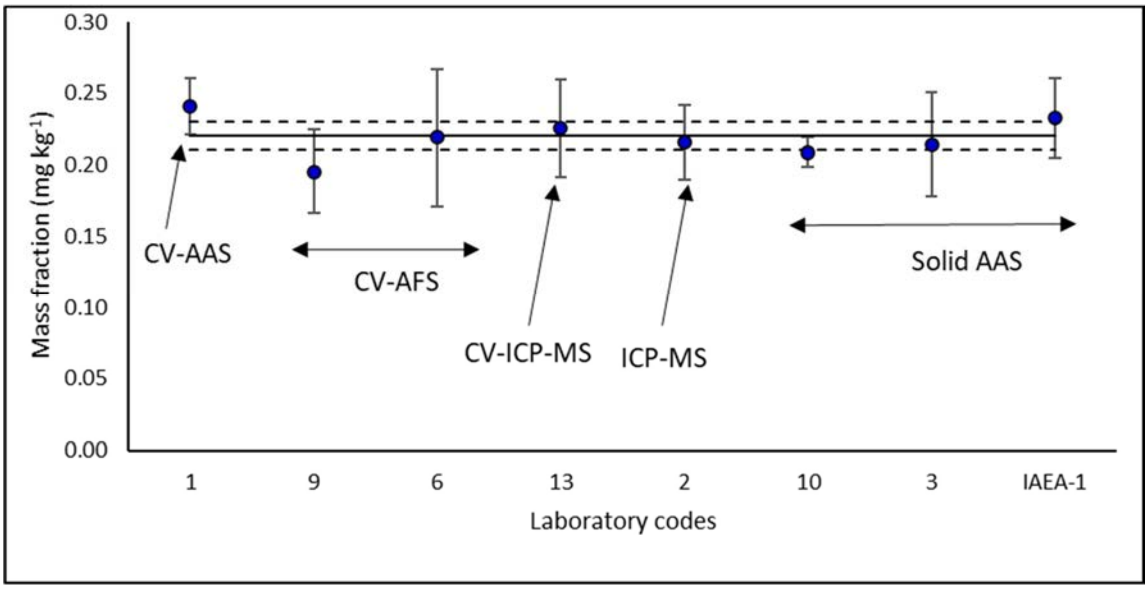

FIG. 53. *Laboratory results for mercury mass fraction (mg kg^{-1}) in IAEA-407A.*

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	12.4	0.9	DORM-4	Neutron Activation
7	11.8	0.9	DORM-4	ICP-MS
9	14.1	0.5	DORM-4	ICP-MS
12	12.7	1.9	IAEA 407	Neutron Activation
IAEA-1	13.3	1.8	IAEA 407	Flame AAS

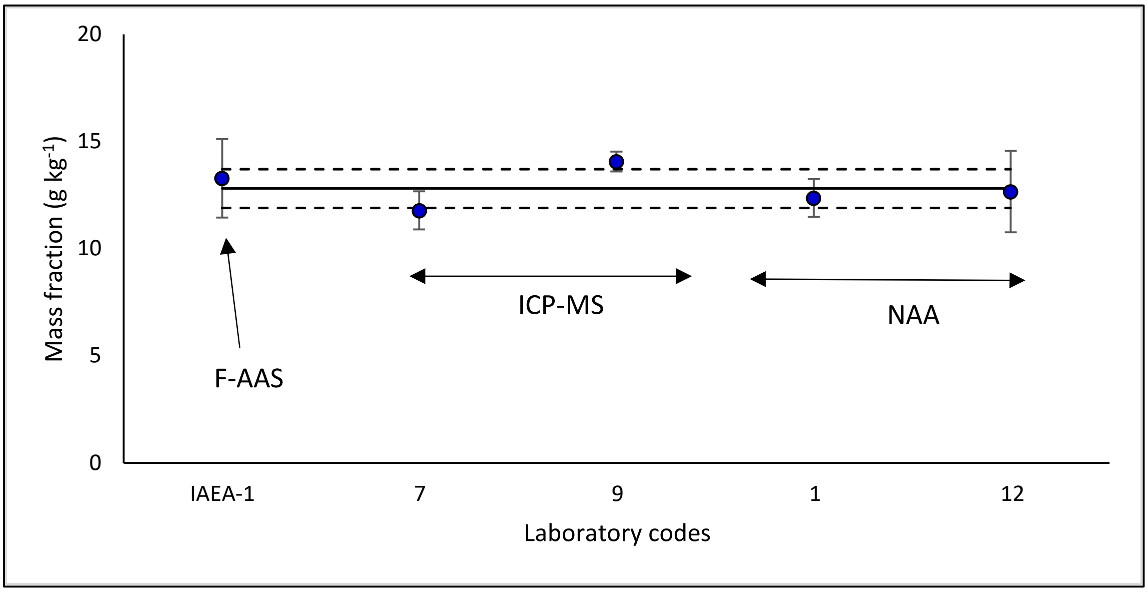

FIG. 54. Laboratory results for potassium mass fraction (g kg^{-1}) in IAEA-407A.

TABLE 14. METHYL MERCURY: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1} as Hg)

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	0.196	0.015	DORM-4	GC-AFS
3	0.188	0.051	DORM-4	GC-AFS
9	0.177	0.056	DORM-4	GC-AFS
IAEA-1	0.195	0.023	IAEA 476	GC-AFS
IAEA-3	0.208	0.010	IAEA 436A	Solid AAS

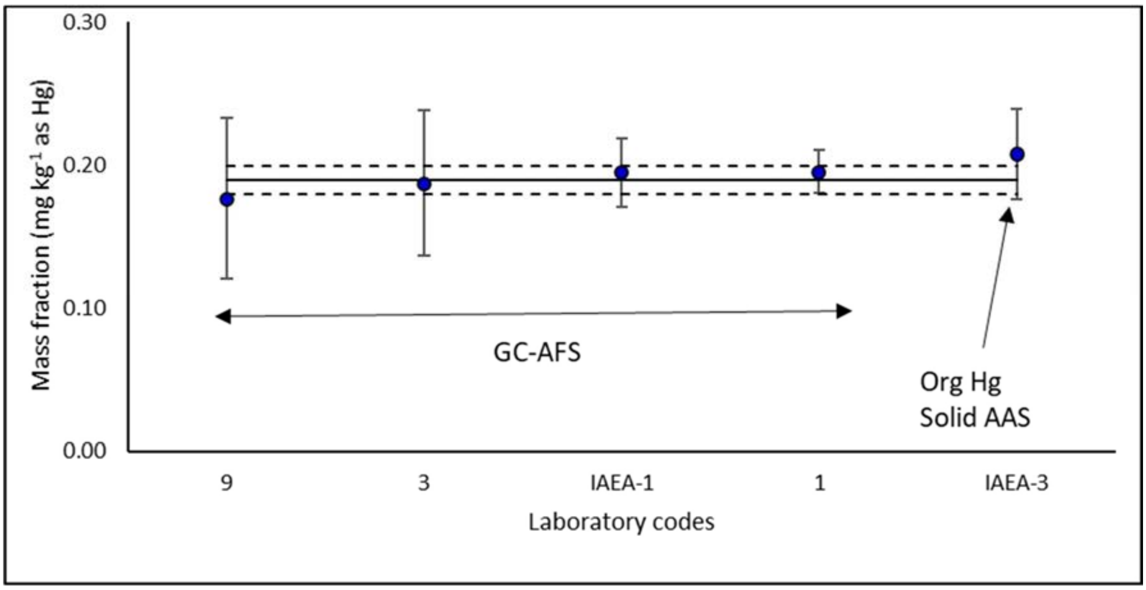

FIG. 55. Laboratory results for methyl mercury mass fraction (mg kg^{-1}as Hg) in IAEA-407A.

TABLE 15. MAGNESIUM: RESULTS AS REPORTED BY PARTICIPANTS (g kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	2.41	0.24	DORM-4	Neutron Activation
4	2.23	0.09	DORM-4	ICP-OES
7	2.17	0.16	DORM-4	ICP-MS
9	2.47	0.14	DORM-4	ICP-MS
12	2.50	0.44	IAEA 407	Neutron Activation
13	2.33	0.35	DORM-4	ICP-MS
IAEA-1	2.64	0.37	IAEA 407	Flame AAS

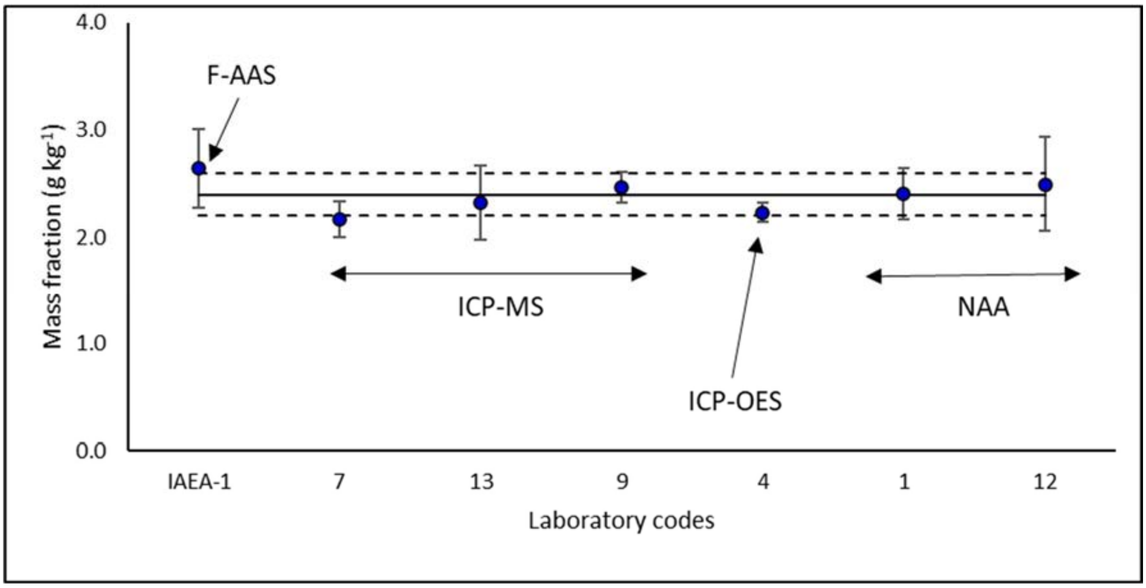

FIG. 56. Laboratory results for magnesium mass fraction (g kg^{-1}) in IAEA-407A.

TABLE 16. MANGANESE: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	3.24	0.32	DORM-4	Neutron Activation
2	3.77	0.44	IAEA 436A	ICP-MS
4	3.79	0.40	DORM-4	ICP-MS
6	3.47	0.49	DORM-4	Flame AAS
7	3.35	0.26	DORM-4	ICP-MS
9	3.70	0.52	DORM-4	ICP-MS
10	4.09	0.27	CE 278K	ICP-MS
12	3.50	0.60	IAEA 407	Neutron Activation
13	3.09	0.46	DORM-2	ICP-MS
IAEA-1	3.64	0.55	IAEA 476	Graphite Furnace AAS
IAEA-2	3.65	0.50	IAEA 476	ICP-MS

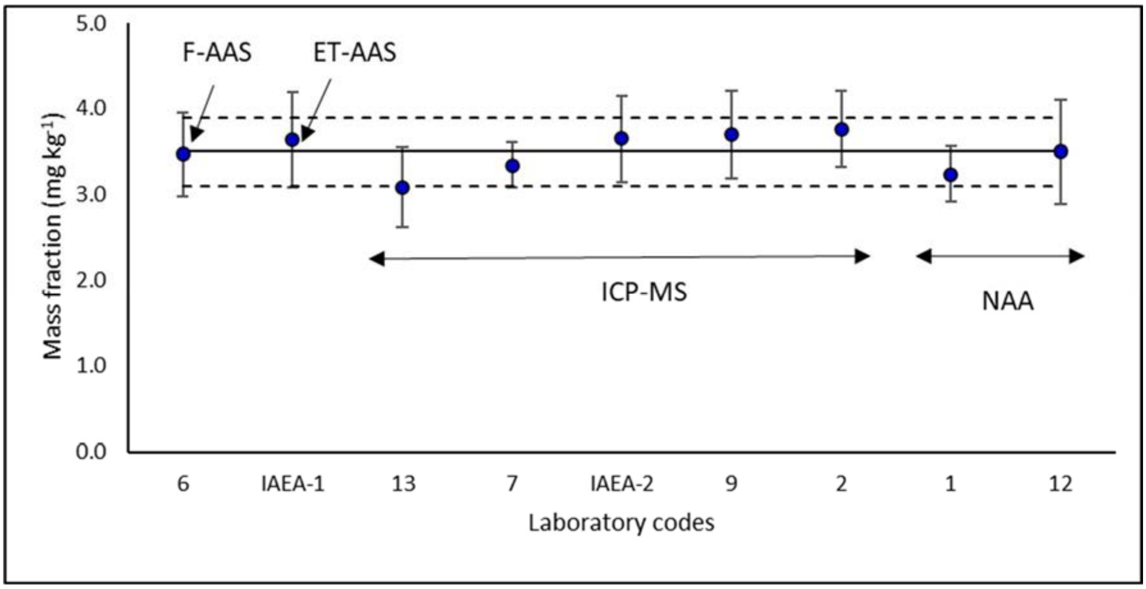

FIG. 57. Laboratory results for manganese mass fraction (mg kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	13.8	1.0	DORM-4	Neutron Activation
4	13.8	0.5	DORM-4	ICP-OES
9	15.2	1.1	DORM-4	ICP-MS
12	13.1	1.6	IAEA 407	Neutron Activation
IAEA-1	14.9	2.2	IAEA 407	Flame AAS

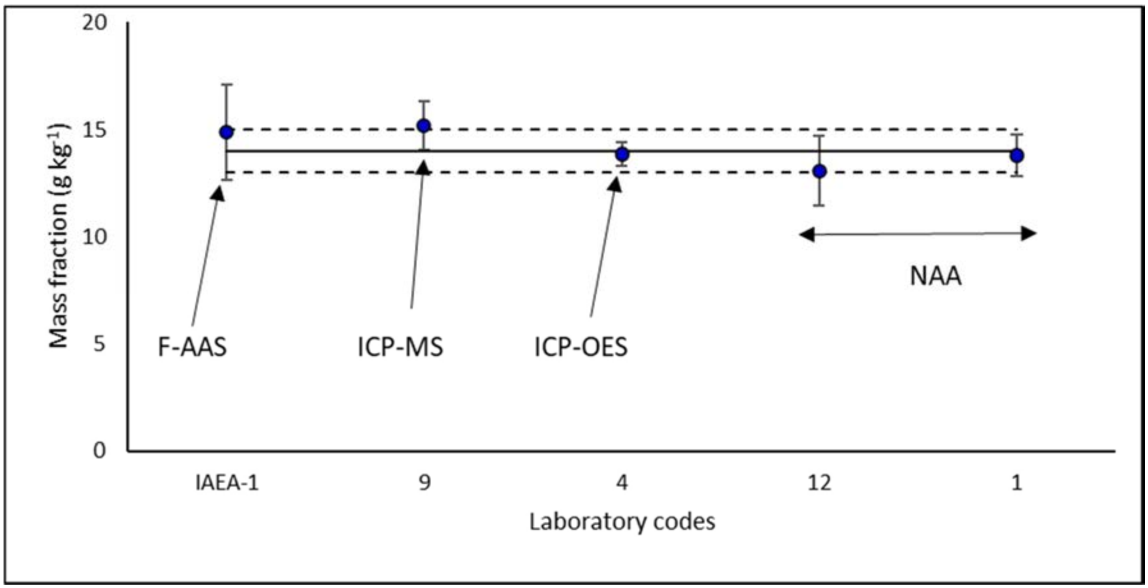

FIG. 58. Laboratory results for sodium mass fraction (g kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	2.39	0.18	DORM-4	Neutron Activation
2	2.56	0.11	IAEA 436A	ICP-MS
7	2.37	0.15	NIST 2702	ICP-MS
9	2.51	0.28	DORM-4	ICP-MS
12	2.38	0.29	SRM 1515	Neutron Activation
IAEA-1	2.55	0.37	IAEA 470	Flame AAS

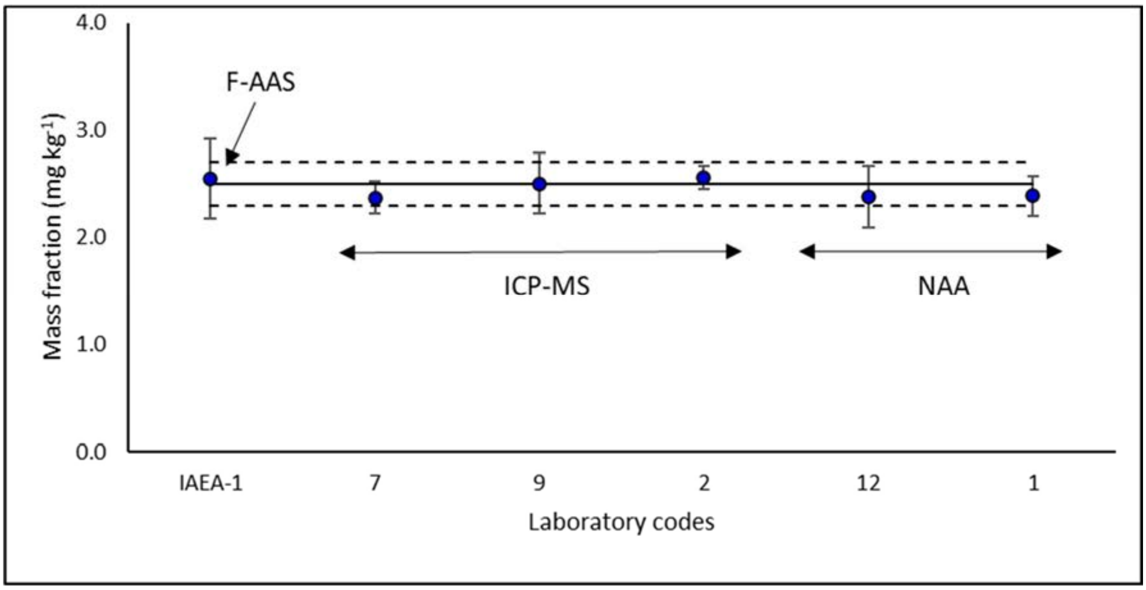

FIG. 59. Laboratory results for rubidium mass fraction (mg kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	2.47	0.18	DORM-4	Neutron Activation
7	2.32	0.09	DORM-4	ICP-MS
9	2.63	0.34	DORM-4	ICP-MS
10	3.58	0.24	CE 278K	ICP-MS
12	3.02	0.37	IAEA 407	Neutron Activation
13	2.55	0.26	DORM-2	ICP-MS
IAEA-2	2.59	0.36	IAEA 476	ICP-MS

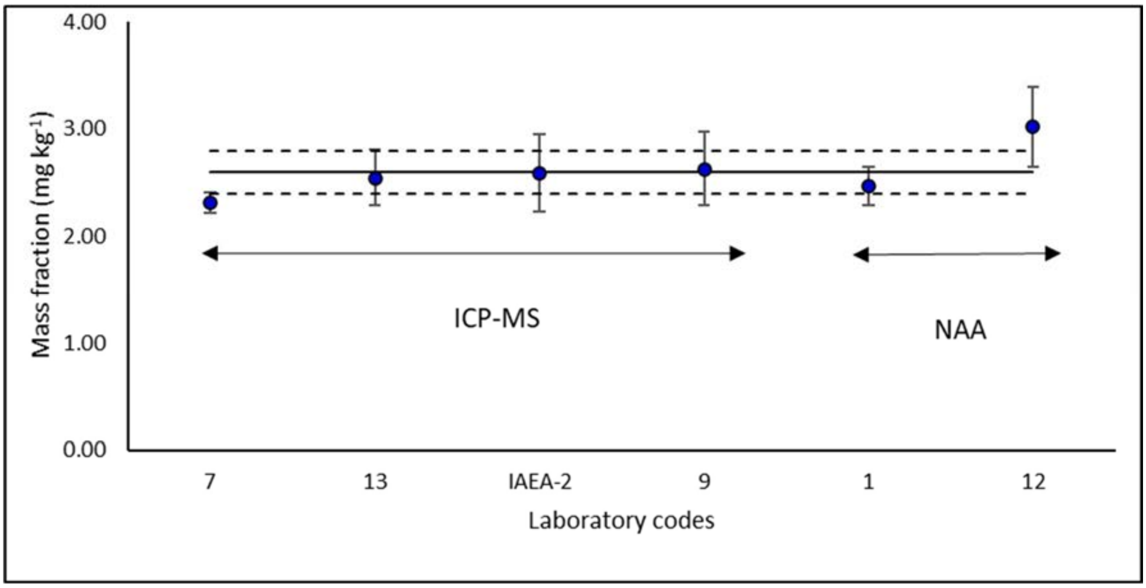

FIG. 60. Laboratory results for selenium mass fraction (mg kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	120	10	DORM-4	Neutron Activation
2	140	6	IAEA 476	ICP-MS
4	122	8	DORM-4	ICP-MS
9	140	17	DORM-4	ICP-MS
12	121	26	IAEA 407	Neutron Activation
13	123	19	DORM-4	ICP-MS
IAEA-1	137	21	IAEA 470	Flame AAS
IAEA-4	124	5		Isotope Dilution ICP-MS

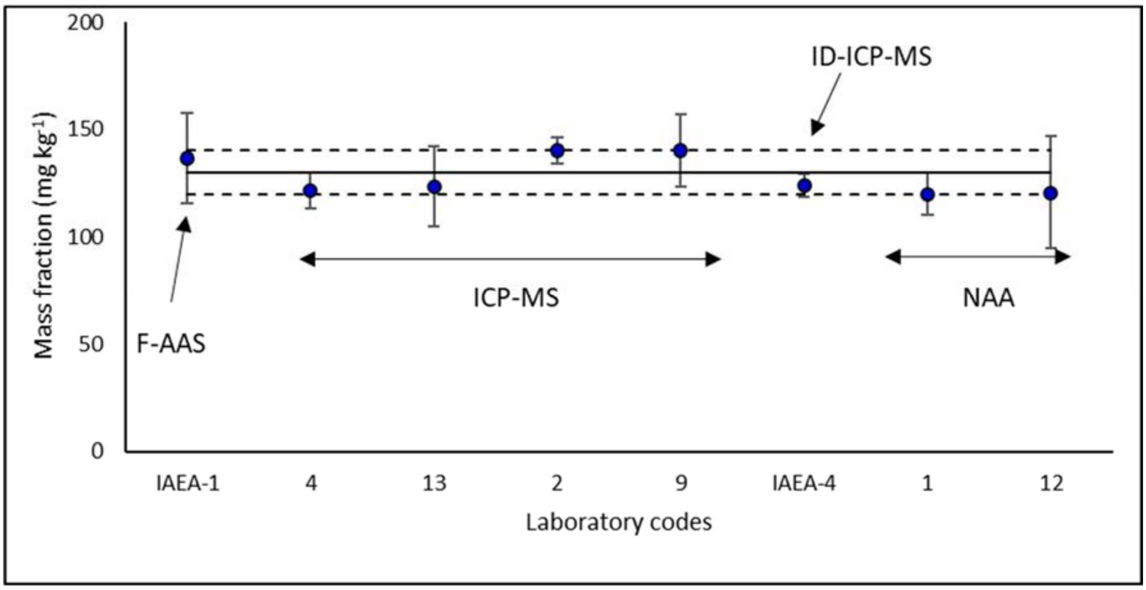

FIG. 61. Laboratory results for strontium mass fraction (mg kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
4	1.47	0.02	DORM-4	ICP-MS
7	1.28	0.05	DORM-4	ICP-MS
9	1.52	0.18	DORM-4	ICP-MS
10	1.68	0.11	IAEA 407	ICP-MS
12	1.40	0.40	IAEA 407	Neutron Activation
13	1.19	0.18	DORM-4	ICP-MS
IAEA-2	1.50	0.21	IAEA 476	ICP-MS

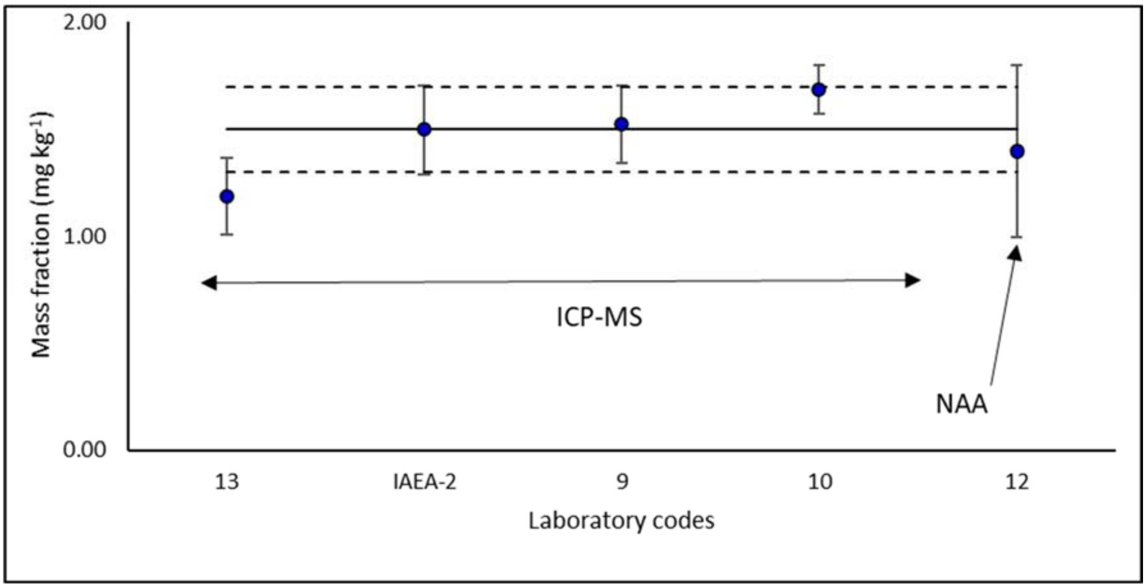

FIG. 62. Laboratory results for vanadium mass fraction (mg kg^{-1}) in IAEA-407A.

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	64.7	4.5	DORM-4	Neutron Activation
2	71.2	3.9	IAEA 436A	ICP-MS
4	58.8	1.4	DORM-4	ICP-MS
6	64.0	10.2	Oyster 1566b	Flame AAS
7	65.8	9.7	DORM-4	ICP-MS
9	63.5	8.3	DORM-4	ICP-MS
10	71.7	4.8	CE 278K	ICP-MS
12	62.0	7.6	SRM 1515	Neutron Activation
13	63.2	9.5	DORM-3	ICP-MS
IAEA-1	67.0	9.4	IAEA 476	Flame AAS
IAEA-2	69.4	9.7	IAEA 476	ICP-MS
IAEA-4	67.0	2.1		Isotope Dilution ICP-MS

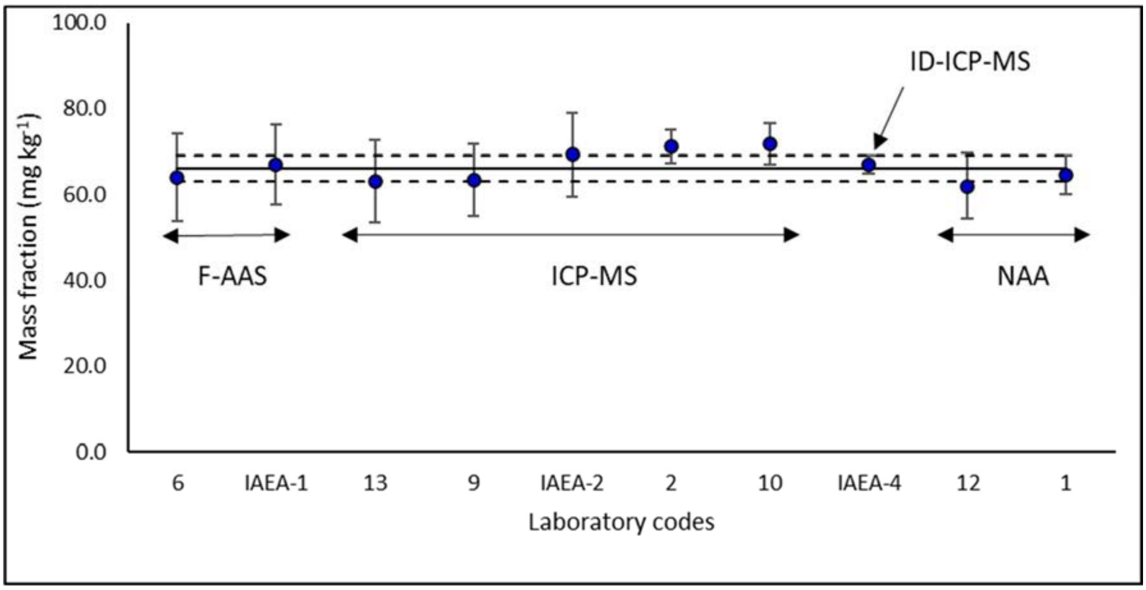

FIG. 63. *Laboratory results for zinc mass fraction (mg kg^{-1}) in IAEA-407A.*

APPENDIX IV

RESULTS OF THE CHARACTERIZATION MEASUREMENTS FOR ELEMENTS WITH INFORMATION VALUES

The results reported by the participating laboratories, their expanded uncertainty, measurement techniques and CRMs used for quality assurance purposes are presented in Tables 23-31. Results not considered after technical evaluation (2.2) are shaded in grey. Figures 64 – 72 provide graphical presentation of the individual results and their expanded uncertainties (*k*=2) as well as the information value for the respective trace element and its expanded uncertainty (*k*=2).

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

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
7	0.037	0.003	DORM-4	ICP-MS
9	0.034	0.007	DORM-4	ICP-MS
10	0.029	0.002	CE 278K	ICP-MS
12	0.053	0.010	IAEA 407	Neutron Activation
13	0.036	0.007	DORM-4	ICP-MS
IAEA-2	0.037	0.006	IAEA 476	ICP-MS

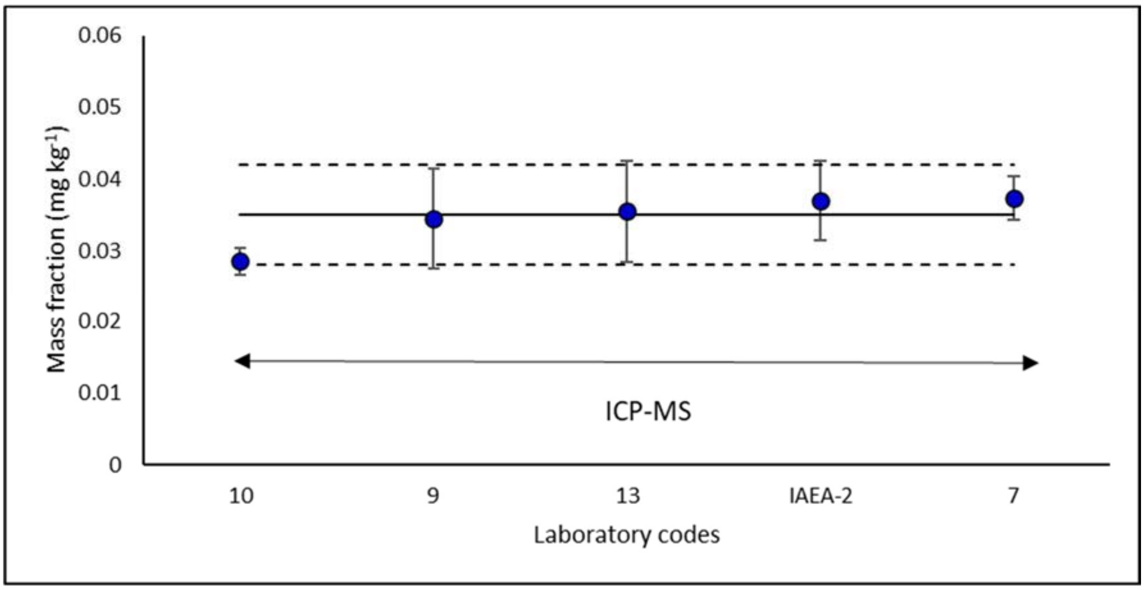

FIG. 64. Laboratory results for silver mass fraction (mg kg^{-1}) in IAEA-407A.

TABLE 24. ALUMINIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
4	6.3	1.0	DORM-4	ICP-OES
7	7.0	1.4	DORM-4	ICP-MS
9	8.8	1.5	DORM-4	ICP-MS
10	15.3	1.0	IAEA 407	ICP-MS
13	15.9	4.8	DORM-2	ICP-MS
IAEA-2	13.4	2.1	IAEA 407	ICP-MS

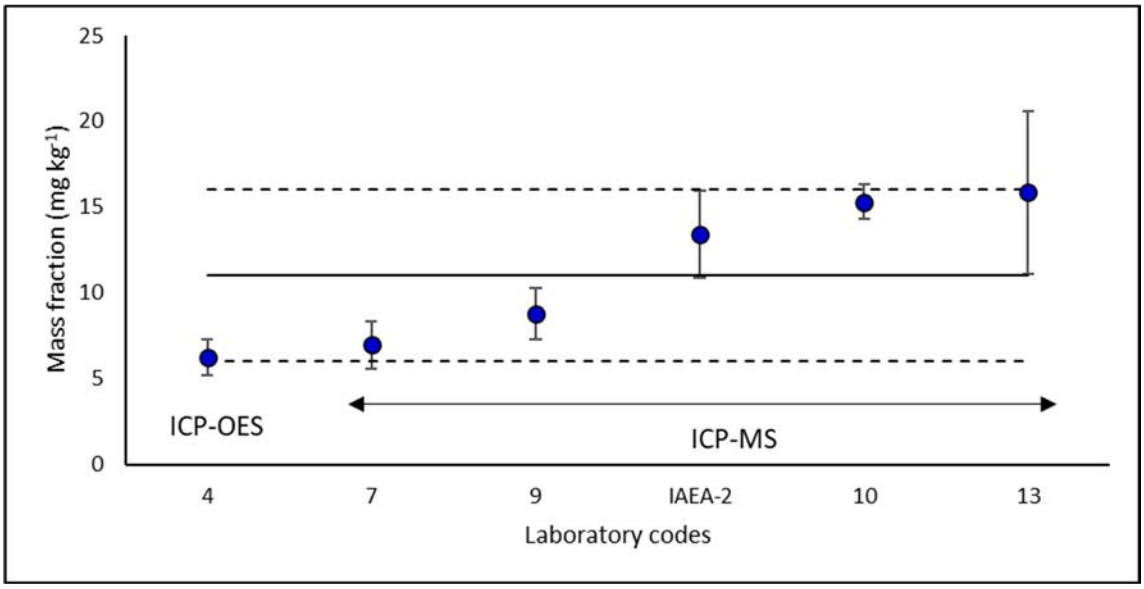

FIG. 65. Laboratory results for aluminum mass fraction (mg kg^{-1}) in IAEA-407A.

TABLE 25. COLBALT: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	0.076	0.010	DORM-4	Neutron Activation
2	0.113	0.012	IAEA 476	ICP-MS
4	0.066	0.006	DORM-4	ICP-MS
7	0.092	0.009	DORM-4	ICP-MS
9	0.078	0.008	DORM-4	ICP-MS
10	0.079	0.005	CE 278K	ICP-MS
12	0.085	0.011	SRM 1515	Neutron Activation
13	0.076	0.015	DORM-2	ICP-MS
IAEA-2	0.125	0.018	IAEA 476	ICP-MS

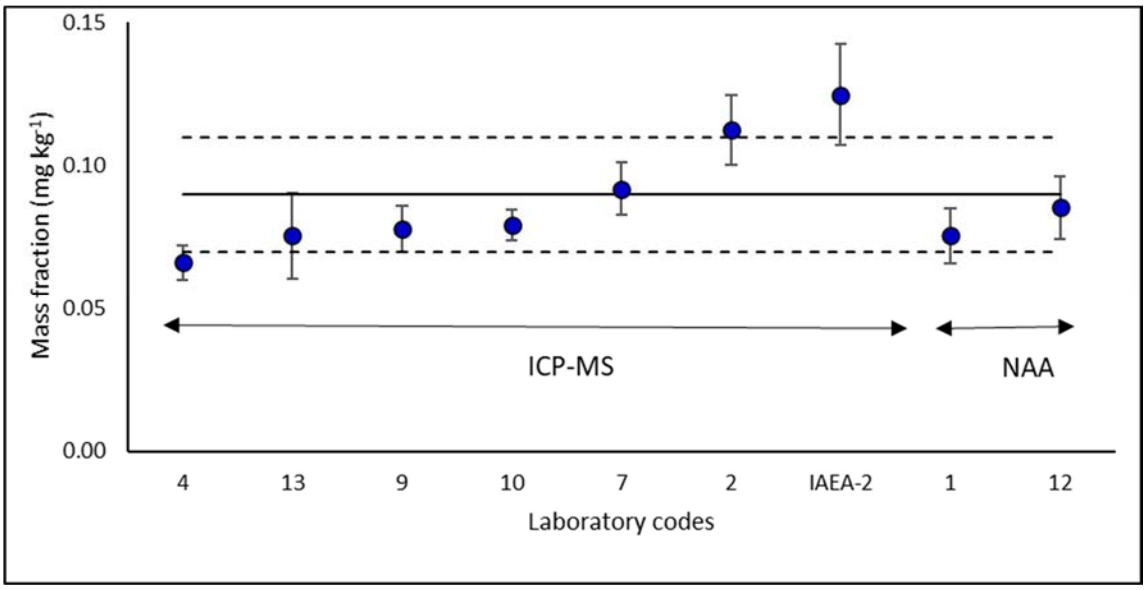

FIG. 66. Laboratory results for cobalt mass fraction (mg kg^{-1}) in IAEA-407A.

TABLE 26. CHROMIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
1	0.577	0.094	DORM-4	Neutron Activation
4	0.698	0.120	DORM-4	ICP-MS
7	0.497	0.097	DORM-4	ICP-MS
9	1.055	0.230	DORM-4	ICP-MS
10	0.766	0.051	IAEA 407	ICP-MS
12	0.646	0.090	IAEA 407	Neutron Activation
13	0.503	0.101	DORM-4	ICP-MS
IAEA-1	0.637	0.100	IAEA 476	Graphite Furnace AAS
IAEA-2	0.669	0.087	IAEA 476	ICP-MS

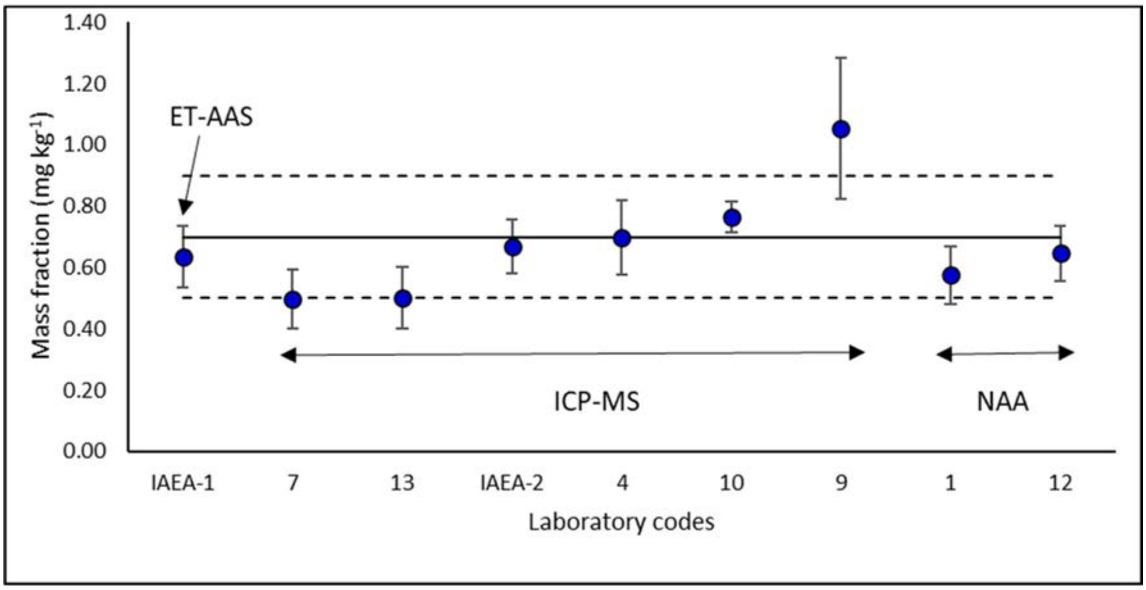

FIG. 67. Laboratory results for chromium mass fraction (mg kg^{-1}) in IAEA-407A.

TABLE 27. LITHIUM: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
7	0.558	0.026	DORM-4	ICP-MS
9	0.637	0.082	DORM-4	ICP-MS
10	0.867	0.058	IAEA 407	ICP-MS
IAEA-2	0.682	0.096	IAEA 407	ICP-MS

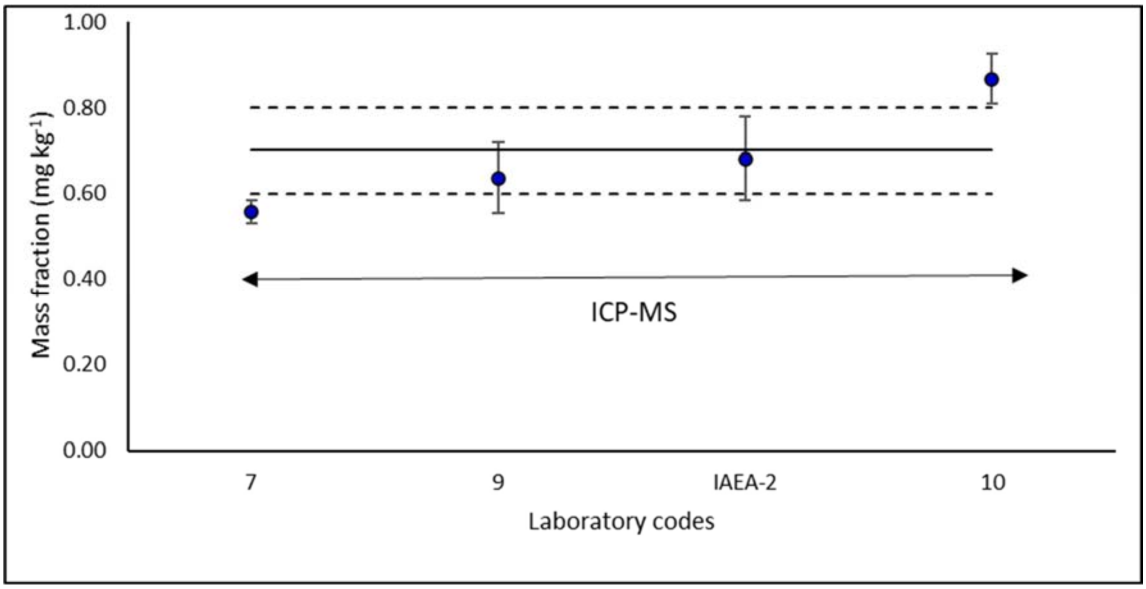

FIG. 68. Laboratory results for lithium mass fraction (mg kg^{-1}) in IAEA-407A.

TABLE 28. NICKEL: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
4	0.314	0.040	DORM-4	ICP-MS
7	0.417	0.037	DORM-4	ICP-MS
9	0.620	0.150	DORM-4	ICP-MS
10	0.521	0.035	CE 278K	ICP-MS
13	0.399	0.799	DORM-3	ICP-MS
IAEA-2	0.462	0.062	IAEA 476	ICP-MS

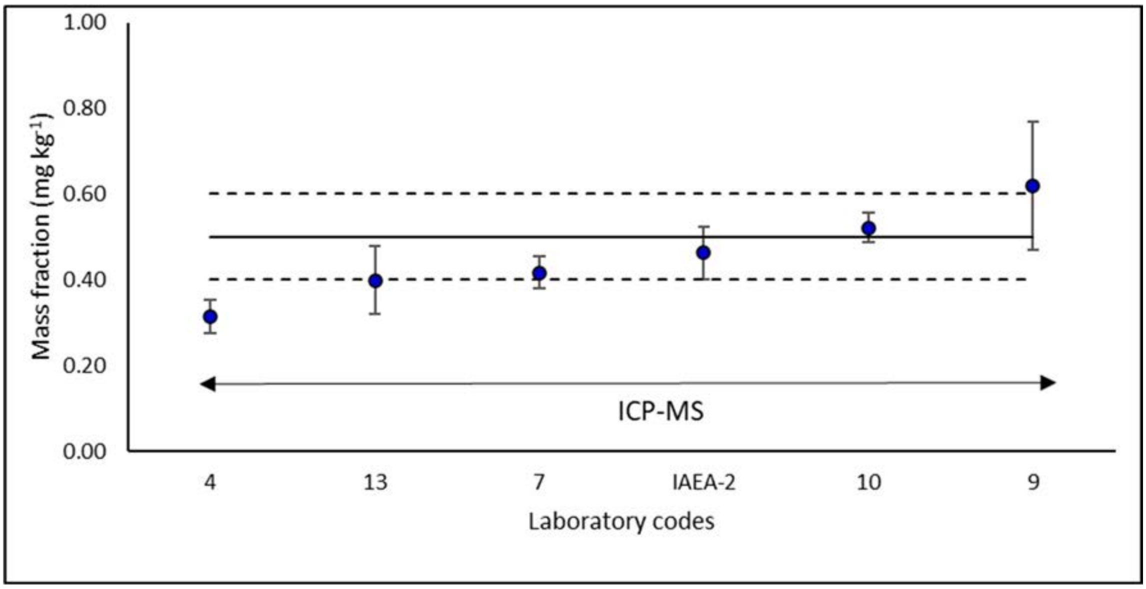

FIG. 69. Laboratory results for nickel mass fraction (mg kg^{-1}) in IAEA-407A.

TABLE 29. LEAD: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
2	0.121	0.019	IAEA 476	ICP-MS
6	0.063	0.013	DORM-4	Graphite Furnace AAS
7	0.090	0.020	NIST 2702	ICP-MS
9	0.088	0.017	DORM-4	ICP-MS
10	0.087	0.006	CE 278K	ICP-MS
12	1.269	0.420	Grass Gr94	XRF
13	0.087	0.017	DORM-4	ICP-MS
IAEA-1	0.106	0.020	IAEA 476	Graphite Furnace AAS
IAEA-2	0.108	0.013	IAEA 476	ICP-MS
IAEA-4	0.119	0.018		Isotope Dilution ICP-MS

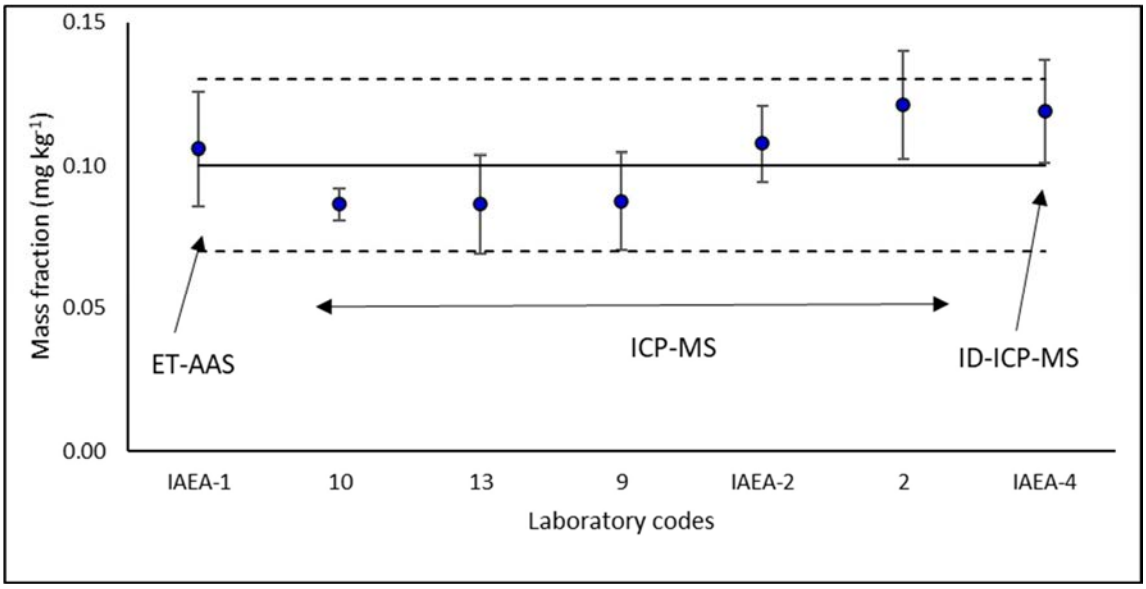

FIG. 70. Laboratory results for lead mass fraction (mg kg^{-1}) in IAEA-407A.

TABLE 30. ANTIMONY: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
4	0.008	0.002	DORM-4	ICP-MS
7	0.014	0.003	NIST 2702	ICP-MS
10	0.009	0.001	IAEA 407	ICP-MS
IAEA-2	0.009	0.001	IAEA 476	ICP-MS

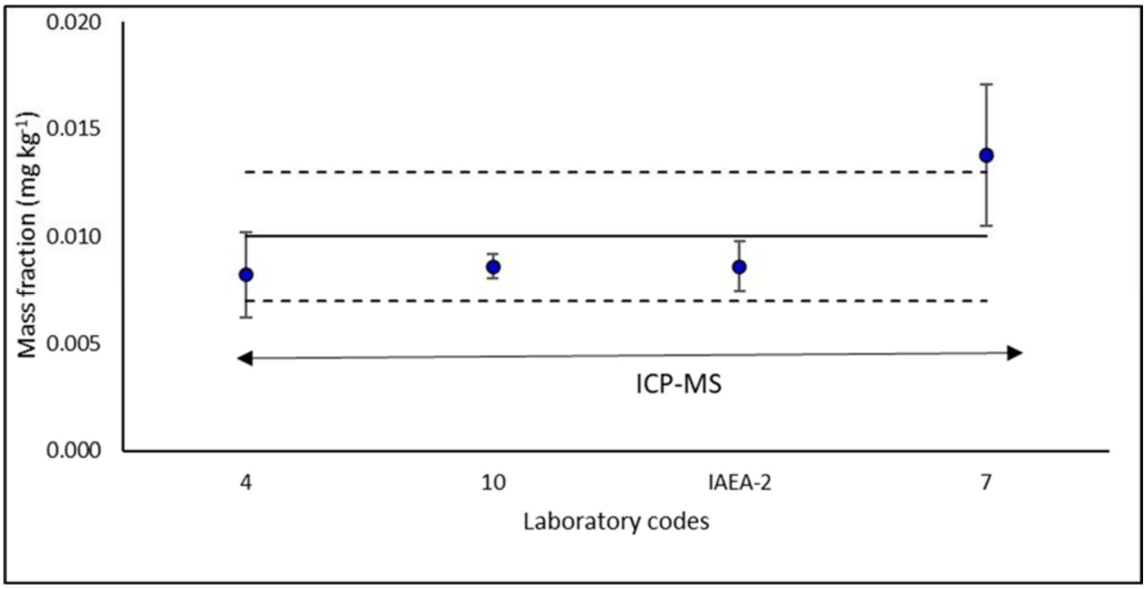

FIG. 71. Laboratory results for antimony mass fraction (mg kg^{-1}) in IAEA-407A.

TABLE 31. TIN: RESULTS AS REPORTED BY PARTICIPANTS (mg kg^{-1})

Laboratory code	Mean	Expanded uncertainty (U)	CRM	Method
7	0.078	0.014	DORM-4	ICP-MS
13	0.070	0.014	DORM-3	ICP-MS
IAEA-2	0.099	0.014	IAEA 407	ICP-MS

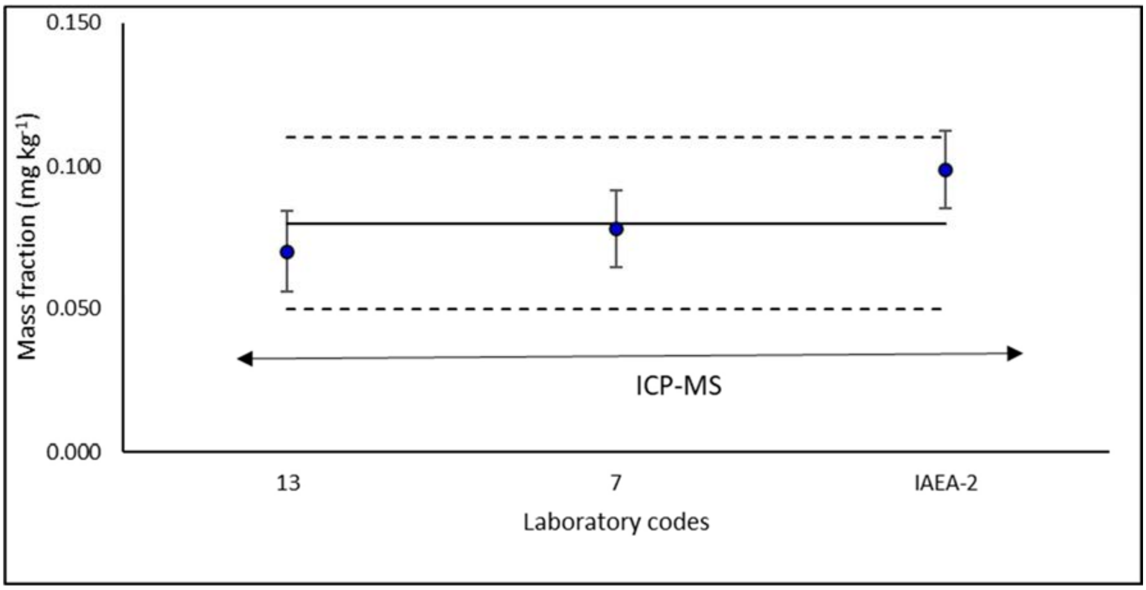

FIG. 72. Laboratory results for tin mass fraction (mg kg^{-1}) in IAEA-407A.

REFERENCES

[1] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories, ISO, Geneva (2017).

[2] UNITED NATIONS ENVIRONMENT PROGRAMME, Minamata Convention Agreed by Nations, Press Release, UNEP, Geneva/Nairobi, (2019).

[3] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, ISO Guide 17034:2016, General requirements for the competence of reference material producers, ISO, Geneva (2016).

[4] INTERNATIONAL ATOMIC ENERGY AGERNCY, World-wide intercomparison exercise for the determination of trace elements and methylmercury in fish homogenate IAEA-407, IAEA/AL/144, IAEA, Vienna (2003).

[5] LINSINGER T., PAUWELS J., VAN DER VEEN A., SCHIMMEL H., LAMBERTY A., Homogeneity and stability of reference materials, Accredit. Qual. Assur. 6 1 (2001) 20–25.

[6] INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, ISO Guide 35:2017, Reference Materials – General and Statistical Principles for Certification, ISO, Geneva (2017).

[7] ORANI A. M., BARATS A., VASSILEVA E., THOMAS O. P., Marine sponges as a powerful tool for trace elements biomonitoring studies in coastal environment, Mar. Pollut. Bull. 131 (2018) 633–645.

[8] ORANI A. M., VASSILEVA E., AZEMARD S., THOMAS O. P., Comparative study on Hg bioaccumulation and biotransformation in Mediterranean and Atlantic sponge species, Chemosphere,260 (2020) 127515.

[9] A. LAMBERTY, H. SCHIMMEL, AND J. PAUWELS, SCHIMMEL H., The study of stability of reference materials by isochronous measurements, Fresenius ' J Anal Chem, 360, (1998) 359–361.

[10] INTERNATIONAL ATOMIC ENERGY AGERNCY, Certification of Trace Elements and Methylmercury Mass Fractions in Fish Homogenate IAEA-476, IAEA, IAEA/AQ/57, IAEA, Vienna (2018).

[11] INTERNATIONAL ATOMIC ENERGY AGERNCY, Worldwide interlaboratory comparison on the determination of trace elements in a fish sample, IAEA, IAEA/AQ/55, IAEA, Vienna (2018).

[12] JOINT COMMITTEE FOR GUIDES IN METROLOGY, Evaluation of measurement data – Guide to the expression of uncertainty in measurement, JGCM 100: 2008 (GUM 1995 with minor corrections), (2008).

[13] VESPER, A., EMONS, H., GNEZDA, M., JAIN, C., MILLER, G., REJ, R., SCHUMANN, G., TATE, J., THIENPONT, L., VAKS, E., Characterization and qualification of commutable reference materials for laboratory medicine; Approved Guideline, CLSI document EP30-A, Clinical and Laboratory Standards Institute,

LIST OF PARTICIPANTS

CANADA

Flett, R.	Flett Research Ltd
Dawn, G.	Flett Research Ltd
Franco, H.	ALS Environmental (Vancouver)

FINLAND

Sara-Aho, T.	SYKE, Finnish Environment Institute

FRANCE

Briant, N.	LBCM – Metal Contaminant Biogeochemistry Laboratory IFREMER
Le Monier, P.	LBCM – Metal Contaminant Biogeochemistry Laboratory IFREMER
Sireau, T.	LBCM – Metal Contaminant Biogeochemistry Laboratory IFREMER

INTERNATIONAL ATOMIC ENERGY AGENCY

Horsky, M.	IAEA Laboratories Seibersdorf
Azemard, S.	IAEA Marine Environmental Studies Laboratory
Vassileva, E.	IAEA Marine Environmental Studies Laboratory

ISRAEL

Herut, B.	Israel Oceanographic & Limnological Research
Segal, Y.	Israel Oceanographic & Limnological Research

PERU

Bedregal, P.	Instituto Peruano de Energía Nuclear Laboratorio de Técnicas Analíticas (TEAN)
Caceres, C.	Instituto Peruano de Energía Nuclear Laboratorio de Técnicas Analíticas (TEAN)
Camacho, N.R.	Instituto Peruano de Energía Nuclear Laboratorio de Técnicas Analíticas (TEAN)
Casa, R.	Instituto Peruano de Energía Nuclear Laboratorio de Técnicas Analíticas (TEAN)
Hidalgo, P.A.M.	Instituto Peruano de Energía Nuclear Laboratorio de Técnicas Analíticas (TEAN)
Llantoy, P.V.	Instituto Peruano de Energía Nuclear Laboratorio de Técnicas Analíticas (TEAN)
Namihas, M.U.	Instituto Peruano de Energía Nuclear Laboratorio de Técnicas Analíticas (TEAN)

SLOVENIA

Begu, E.	Jozef Stefan Institute Dept. of Environmental Sciences
Horvat, M.	Jozef Stefan Institute Dept. of Environmental Sciences
Jacimovic, R.	Jozef Stefan Institute Dept. of Environmental Sciences

UNITED KINGDOM

Clough, R.	School of Geography, Earth and Environmental Science University of Plymouth

UNITED STATES OF AMERICA

Goodall, A.	Brooks Applied Labs

CONTRIBUTORS TO DRAFTING AND REVIEW

Azemard, S.	International Atomic Energy Agency
Camin, F.	International Atomic Energy Agency
Horsky, M.	International Atomic Energy Agency
Pham, M.	International Atomic Energy Agency
Vasileva-Veleva, E.	International Atomic Energy Agency

ORDERING LOCALLY

IAEA priced publications may be purchased from the sources listed below or from major local booksellers.

Orders for unpriced publications should be made directly to the IAEA. The contact details are given at the end of this list.

NORTH AMERICA

Bernan / Rowman & Littlefield

15250 NBN Way, Blue Ridge Summit, PA 17214, USA
Telephone: +1 800 462 6420 • Fax: +1 800 338 4550

Email: orders@rowman.com • Web site: www.rowman.com/bernan

REST OF WORLD

Please contact your preferred local supplier, or our lead distributor:

Eurospan Group

Gray’s Inn House
127 Clerkenwell Road
London EC1R 5DB
United Kingdom

Trade orders and enquiries:

Telephone: +44 (0)176 760 4972 • Fax: +44 (0)176 760 1640
Email: eurospan@turpin-distribution.com

Individual orders:

www.eurospanbookstore.com/iaea

For further information:

Telephone: +44 (0)207 240 0856 • Fax: +44 (0)207 379 0609
Email: info@eurospangroup.com • Web site: www.eurospangroup.com

Orders for both priced and unpriced publications may be addressed directly to:

Marketing and Sales Unit
International Atomic Energy Agency
Vienna International Centre, PO Box 100, 1400 Vienna, Austria
Telephone: +43 1 2600 22529 or 22530 • Fax: +43 1 26007 22529
Email: sales.publications@iaea.org • Web site: www.iaea.org/publications

22-04789E

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA