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PRACTITIONER'S REPORT

Determination of the reference value of nitrogen mass fraction in the reference material of Polish soil

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Abstract Ensuring a traceability and meaningful of a measurements is one of the most important stages of each analysis, each measurement. It is especially important for measurement of the environmental samples, like soil, which is a very complex matrix. A certified reference materials (CRMs) should be routinely used for this purpose. The paper discusses the procedure for preparation of the soil samples for certification as CRM. As for agricultural reasons there is a growing demand for CRMs regarding a nitrogen mass fraction in the Polish soil, we prepared such a material and established the reference value with associated measurement uncertainty. Homogeneity and stability of the material were shown to be appropriate for the intended purpose. The presented approach can also be used in a process of manufacture of a laboratory reference material, which can be used for a routine quality control.

Keywords Quality control · Reference materials · Homogeneity · Stability · Reference value

Introduction

The use of reference materials (RMs) in analytical measurement is an important part of the reliability of the control of measurement results [1]. RMs can be used for calibration, quality control, method validation or assignment of values to other materials [2]. Reference materials should be properly selected to reflect the real sample. The content of analyte should be similar to the real content.

The most important features of the reference material are homogeneity and stability. Homogeneity is determined on the basis of comparison of the results for randomly selected samples. It is necessary to define within-sample and between-samples homogeneity. Minimum weight of the sample required for the measurements should be defined as well. The degree of homogeneity of the material ensures the recovery of the reference value with the associated uncertainty [1].

Stability of the RM depends on many factors that include the environmental factors (e.g., temperature, humidity, pressure), the way of the transportation and the storage conditions. Stability ensures that the value of the determined property is constant during the declared validity. Proceeding in accordance with the instruction of reference material provided by the producer is necessary to keep the homogeneity and stability [1, 3].

Certified reference materials (CRM) have one or more specified property values with the associated uncertainty and reference to the measurement traceability [4]. For the certified matrix reference material such as soil, the certified reference value can be determined in different ways: by making measurements in a laboratory using a primary reference measurement procedure; based on interlaboratory comparisons using one or several methods or making measurements in a single laboratory using two or more methods used by two or more analysts. In any case, the reference value must be stated with the measurement uncertainty [3].

In the modern fertilizers consulting, to optimize fertilization, a mass fraction of nitrogen input is exactly adapted

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to the type of the cultivation and the mass fraction of this component in the soil. This optimization is possible only if the mass fraction of nitrogen in the soil each time before fertilization is determined. Dosage control is necessary for the proper cultivation of the plants. The value of the different forms of nitrogen in the soil is varied as a result of transformation associated with mineralization, nitrification, denitrification and leaching. The control of the mass fraction of nitrogen in the soil prevents adverse impacts on the environment of this element.

Laboratories, accredited by the Polish Centre for Accreditation (http://www.pca.gov.pl/en/), which have in their scope of accreditation the methods for the determination of nitrogen are required to maintain the measurement traceability according to the ISO/IEC 17025:2005 [5]. According to the Vocabulary in Metrology (VIM) [4], measurement traceability is defined as "property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty." In practice, ensuring the traceability means, among others, that the measurement equipment, which has a significant impact on the uncertainty of measurement, should be calibrated by the National Metrology Institutes and Designated Institutes or accredited calibration laboratories, comparison of individual measurement result relative to a higher-order standard and use of CRMs for the calibration of measuring instruments [6]. It is very important to have the CRM with a matrix similar to the one of the samples to be analyzed. The certified reference value of nitrogen content in commercially available CRMs for the soil matrix is an important selection criteria of CRM in environmental analysis.

In The International Database for CRMs, COMAR 105 CRMs of soil matrix are registered. Only a small part of CRMs of a soil matrix available in this base, Chinese production mainly, has the certified value of the nitrogen mass fraction [7]. Nitrogen is not on the list of the usually analyzed elements of The National Institute of Metrological Research (INRiM) in Italy as well [8].

High-quality RMs are demanding and costly to produce. It is not normally cost effective for laboratories to make their own RMs, so-called laboratory reference materials (LRMs) or in-house reference materials (IHRMs), which are prepared by users for their own purposes [9, 10].

The measurements were taken in framework of the project "Production and attestation of new types of reference materials crucial for achieving European accreditation for Polish industrial laboratories" attributed by the National Center for Research and Development in Poland. The aim of the project was to produce six CRMs, including soil. The soil was prepared for certification of aromatic hydrocarbons and polychlorinated biphenyls content by

interlaboratory comparisons. For this project soil was taken from the areas of the Lower Silesia, Poland. Given the fact that nitrogen content in soil is an important parameter, part of the prepared material was used to provide the reference value of the total nitrogen mass fraction and associated measurement uncertainty.

Experimental procedures

The 150 kg of soil was collected from areas located in the vicinity of the chemical plant in Brzeg Dolny, Lower Silesia, Poland. The soil was air dried, homogenized and screened through a sieve of 250 µm at first. Finally, the soil was brought to a grain size of 100 µm. In a subsequent step, the material was distributed into the amber glass bottles with the screw cap in 50 g portions. All bottles with soil were sterilized by electron beam radiation dose of 28 kGy. The elemental composition and water content were measured. The elemental composition (except nitrogen—N and carbon—C) was determined after extraction in aqua regia with the microwave digestion system Ethos One, Milestone, Italy, using ICP-OES method (inductively coupled plasma–optical emission spectrometry) iCAP 6500 Duo, Thermo Scientific, USA and ICP-MS method (inductively coupled plasma–mass spectrometry) XSeries 2, Thermo Scientific, USA, according to the internal measurement procedures of the accredited laboratory (Accreditation number 696) [11]. N (total nitrogen) and C (total carbon) mass fraction were determined using the elemental analyses method with the analyzer Vario Macro Cube, Elementar Analysensysteme GmbH, Germany. The water content was determined using the gravimetric method (the sample was dried constant mass at 105 °C), according to the ISO 11465:1993/AC1:1994 [12]. The elemental composition and water content of the soil sample are shown in Table 1.

The elemental composition and water content in air-dried soil sample were typical for Polish soil [13].

The soil was packed into glass jars, each containing 50 g of the sample. Ten randomly selected jars, which were marked from F1 to F10, were selected for measurement.

The analyses and statistical evaluation were performed in Chemical Laboratory of Multielemental Analysis, Wrocław University of Technology, Poland, accredited by the Polish Centre for Accreditation (AB 696). Laboratory has in its scope of accreditation two measurement procedures of determination of the nitrogen mass fraction in the soil, which have been validated and are subjected to systematic control of measurement traceability in accordance with the requirements of ISO/IEC 17025:2005 [5]. Both these methods—determination of total nitrogen using titration method, according to the ISO 11261:1995 [14]

Table 1 Elemental composition[a] and water content of the soil

Element	Unit	Mass fraction with associated uncertainty[b]
Al		1.8 ± 0.4
Ca		1.1 ± 0.2
Fe		0.95 ± 0.19
K		0.50 ± 0.10
Mg		0.29 ± 0.06
Mn		0.0380 ± 0.0057
Na	g/(100 g)	0.26 ± 0.05
P		0.10 ± 0.02
S		0.044 ± 0.006
Si		35 ± 7
Ti		0.132 ± 0.026
C		3.5 ± 0.4
N		0.27 ± 0.03
Co		2.9 ± 0.4
Cr		61 ± 9
Cu		27 ± 4
V		17 ± 3
Zn		180 ± 27
As		2.6 ± 0.7
Ba	mg/kg	526 ± 79
Be		<0.50
Cd		0.84 ± 0.13
Hg		0.86 ± 0.13
Ni		18 ± 3
Pb		48 ± 7
Water content	g/g	0.0102 ± 0.0003

[a] Values of elemental composition are based on the dried sample

[b] Expanded uncertainty of the standard method at confidence level 95% (for $k = 2$)

(digestion and distillation system Büchi, Switzerland) and the determination of total nitrogen mass fraction by dry combustion, according to the ISO 13878:2002 [15] (elemental analyzer Vario Macro Cube)—were used to determine the nitrogen mass fraction in the soil samples. Analysis with the use of each of these methods was performed by two analysts.

Between-samples homogeneity

Evaluation of homogeneity aims to confirm that the differences between the individual samples are not systematic and are not statistically significant. The measurements were taken for all ten samples (g − number of samples = 10). Two portions were prepared from each sample ($2g = 20$). On the basis of the obtained results of the analysis,

parameters for the statistical evaluation of homogeneity were determined, in accordance with ISO 13528:2005 [16]. The standard deviation for proficiency testing was estimated on the basis of a general model of Horwitz equation as:

$$\hat{\sigma} = \frac{R \cdot \overline{X}}{100} \tag{1}$$

where R was described by Horwitz as:

$$R = 2^{(1 - 0.5 \cdot \log C)} \tag{2}$$

where $\hat{\sigma}$ is the standard deviation for proficiency testing, R relative standard deviation (RSD), $\overline{X}$ average value of measurements of ten samples of soil in duplicate, C mass fraction (e.g., if the mass fraction is 10 mg/(100 g) then $C = 10 \times 10^{-3}$ g/(100 g) $= 10^{-4}$).

Within-sample homogeneity

In order to evaluate the influence of heterogeneity within a single sample, the evaluation of within-samples homogeneity was conducted. Among the ten selected samples, one jar was randomly selected. From the selected jar, two samples were collected. Two portions were prepared from each sample.

Stability

Stability was evaluated by performing the analysis of three randomly selected samples ($g = 3$), from the ten samples selected to evaluate homogeneity. Two portions were prepared from each sample ($2g = 6$). The measurements were taken at scheduled intervals—after 2 and 4 weeks and 6 months. The procedure of the evaluation of stability was carried out on the basis of the guidelines of ISO 13528:2005 [16]. The same analytical methods as for the homogeneity check were applied. The measurements were taken under repeatability conditions.

In the next step, the impact of the storage temperature on the stability samples was examined. Three randomly selected samples were stored for 6 months in the controlled condition, at +3 °C and −24 °C.

Determination of the reference value

The measurements were taken using two methods of measurement by two analysts. Each analyst performed two series of measurements, ten measurements for each series, both methods. For statistical evaluation 80 measurements were obtained. To detect outliers in a dataset, the Grubbs test was used, according to the ISO 5725-2:1994/AC1:2002 [17]. The reference value was estimated as the average of 80 measurements.

Uncertainty of the reference value

On the basis of all performed measurements, the combined standard uncertainty of reference value was obtained by combining the individual standard uncertainties [18]:

$$u_c = \sqrt{(u_{av})^2 + (u_{bsh})^2 + (u_{wsh})^2 + (u_{lts})^2 + (u_{sts})^2} \quad (3)$$

where u_c uncertainty of the reference value of nitrogen mass fraction, u_{av} uncertainty of determining of the average value of nitrogen mass fraction, u_{bsh} uncertainty associated with the between-samples homogeneity, u_{wsh} uncertainty associated with the within-samples homogeneity, u_{lts} uncertainty associated with long-term stability, u_{sts} uncertainty associated with short-term stability.

Results and discussion

The homogeneity and stability measurements were preceded by the comparison of the nitrogen mass fraction determined by the two different methods (titration method and dry combustion method). The measurements were taken by two analysts. The average of the nitrogen mass fraction determined using both methods is shown in Fig. 1.

In order to evaluate the influence of heterogeneity within a single sample for the result of the measurements, evaluation of the within-sample homogeneity was conducted. The results of the nitrogen mass fraction using both methods conducted by two analysts are shown in Fig. 2.

In the next step, the between-samples homogeneity and the within-sample homogeneity of the soil samples were evaluated. The obtained values of the derived parameters are shown in Table 2. Adopted criterion of homogeneity: $S_S \leq 0.3\hat{\sigma}$. Additional data are accessible as Electronic Supplementary Material (ESM).
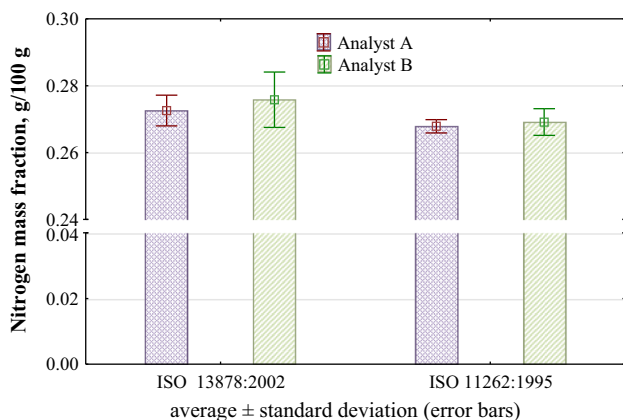
Fig. 1 Average of the nitrogen mass fraction determined by the Kjeldahl method and dry combustion method
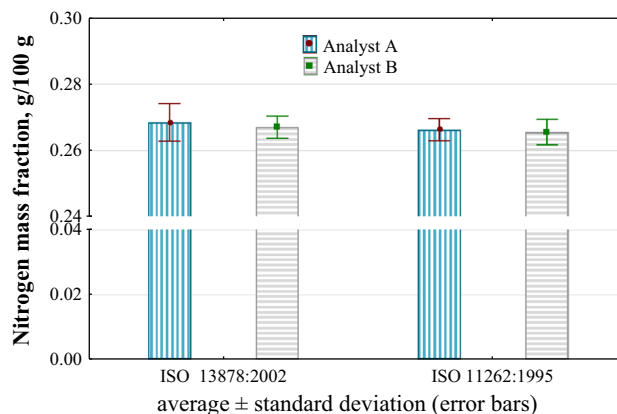
Fig. 2 Average of the nitrogen mass fraction within the single sample

Results of the evaluation of the homogeneity showed that the standard deviation between the samples was less than the criterion of homogeneity check; therefore, the given samples can be considered as sufficiently homogeneous.

In the case of the standard deviation between the samples collected from the same jar, received as a result of the measurement of the within-sample homogeneity, the criterion of homogeneity was also met; therefore, the given samples can be considered as sufficiently homogeneous within one sample.

It should be remembered, that the material is homogeneous for a specific mass of the sample, for which the homogeneity was verified. The material can be homogeneous when a large mass of material is used for analysis, but heterogeneity may be detectable in small masses of sample [19]. The minimum weight of sample recommended for the use should be specified on the reference material certificate. All the measurements were taken on the soil samples with the mass of 200 mg, therefore for the given material the samples minimum measurement portion having the mass of 200 mg.

On the basis of the results, the evaluations of the short-term and long-term stability were made. Adopted criterion of stability: $|\overline{X} - \overline{Y}| \leq 0.3\hat{\sigma}$. The results are shown in Table 3.

In the next step, the impact of the storage temperature on the stability of the samples was examined. Adopted criterion of stability: $|\overline{X} - \overline{Y}| \leq 0.3\hat{\sigma}$. The results are shown in Table 4.

The stability of the material was verified after the homogeneity of the soil samples was confirmed. The stability criterion for the samples was met. The obtained results indicated that the material was stable under the studied conditions. The material can be stored in the fridge and can be frozen.

The reference value was determined based on the measurement results of the ten soil samples. The average

Table 2 Homogeneity of nitrogen mass fraction, g/(100 g)

Parameter	Symbol	Between-samples homogeneity	Within-samples homogeneity
The general average defined as: $\overline{X} = \left(\sum_{t=1}^{g} \overline{X}_t\right) \Big/ g$ where X_t result of the measurement of portion	$\overline{X}$	0.271	0.266
The sum of the differences of between-test-portion ranges	ΣW_t	0.0500	0.0237
Within-samples standard deviation $S_w = \sqrt{\left(\sum_{t=1}^{g} W_t^2\right) \Big/ (2g)}$	S_w	0.0030	0.0015
Standard deviation of sample average $S_X = \sqrt{\left(\sum_{t=1}^{g} (\overline{X}_t - \overline{X})^2\right) \Big/ (g-1)}$	S_x	0.0027	0.0028
Between-samples standard deviation $S_S = \sqrt{S_X^2 - (S_w^2/2)}$	S_s	0.0016	0.0026
Standard deviation for proficiency testing—Eq. (1)	$\hat{\sigma}$	0.013	0.013
	$0.3\hat{\sigma}$	0.0039	0.0039
Evaluation of the homogeneity		Homogeneity criterion met because $S_S < 0.3\hat{\sigma}$	

Table 3 Short-term and long-term stability

Parameter	Symbol	Value, g/(100 g)		
General average of the measurements obtained in the homogeneity check	$\overline{X}$	0.2710		
Standard deviation for proficiency testing—Eq. (1)	$\hat{\sigma}$	0.0130		
	$0.3\hat{\sigma}$	0.0039		
Results of the evaluation of stability		After 2 weeks, g/(100 g)	After 4 weeks, g/(100 g)	After 6 months, g/(100 g)
General average mass fraction of the results obtained in the stability check $\overline{Y} = \left(\sum_{t=1}^{g} \sum_{p=1}^{2} Y_{tp}\right) \Big/ (2g)$ where Y_{tp} is the measurement result for sample t and portion p	$\overline{Y}$	0.2671	0.2672	0.2749
The difference between the average values	$\left|\overline{X} - \overline{Y}\right|$	0.0039	0.0038	0.0039
Evaluation of the stability		Stability criterion met because $\left|\overline{X} - \overline{Y}\right| \leq 0.3\hat{\sigma}$		

values of the measurements are shown in Table 5, separately for the each analyst and each measurement method. Additional data are accessible in the ESM.

The average nitrogen mass fraction determined using the modified Kjeldahl method was 0.266 g/(100 g), and the thermo-conductometric method 0.268 g/(100 g), what represents the difference at the level of approximately 0.8%. The same two methods were used by Gawlik et al.

[20] to determine the mass fraction of total nitrogen in the study of certification of the European Reference Soil Set, and comparable results were achieved. It might confirm usefulness and relevance of use of these two methods for the analysis of the total nitrogen in the soil samples.

The reference values of the nitrogen mass fraction in the given material were developed from the analytical data obtained from the analysis performed with the two,

Table 4 Influence of temperature (T) on the stability of the soil samples

Parameter	Symbol	Value, g/(100 g)	
General average of the measurements obtained in the homogeneity check	$\overline{X}$	0.2710	
Standard deviation for proficiency testing—Eq. (1)	$\hat{\sigma}$	0.0130	
	$0.3\hat{\sigma}$	0.0039	
Results of the evaluation of stability		At $T = 3$ °C, g/(100 g)	At $T = -24$ °C, g/(100 g)
General average of the results obtained in the stability check	$\overline{Y}$	0.2677	0.2676
$\overline{Y} = \left(\sum_{t=1}^{g} \sum_{p=1}^{2} Y_{tp} \right) \Big/ (2g)$ Y_{tp} as in Table 3			
The difference between the average values	$\left| \overline{X} - \overline{Y} \right|$	0.0033	0.0034
Evaluation of the stability		Stability criterion met because $S_S < 0.3\hat{\sigma}$	

Table 5 Reference value of the nitrogen mass fraction in the soil sample

Average value of nitrogen mass fraction, g/(100 g)		
	Analyst A	Analyst B
According to ISO 13878	0.266	0.266
According to ISO 11261	0.268	0.267

The reference value of nitrogen mass fraction in the soil sample is 0.267 g/(100 g)

Table 6 Uncertainty of the reference value of the nitrogen mass fraction in the soil sample; all data in g/(100 g)

Standard uncertainty[a] of the components	
Determining of the average value, u_{av}	0.0037
Between-samples homogeneity, u_{buh}	0.0027
Within-samples homogeneity, u_{wuh}	0.0028
Long-term stability, u_{lts}	0.0061
Short-term stability, u_{sts}	0.0052
Combined standard uncertainty, u_c	**0.010**
Expanded uncertainty[b]	**0.020**
Reference value of the nitrogen mass fraction	**0.267 ± 0.020**

[a] Expressed as the standard deviation of the result obtained using both measurement procedures

[b] Coverage factor $k = 2$

independent methods, used by two analysts. According to the ISO Guide 35:2006, this approach is technically correct and reasonable [21]. In the given soil sample, the reference value was set at 0.2667 g/(100 g) of the nitrogen. According to the latest data published by Chief Inspectorate of Environmental Protection in Poland, the nitrogen mass fraction in the Polish soils is at the range of 0.04–0.41 g/(100 g) [22]. The total nitrogen mass fraction in the given sample was therefore typical for Polish soils. This confirmed that the natural matrix of the CRM was prepared from the material found in nature [13].

In the final stage of the research, the uncertainty of the reference value was determined. The standard combined uncertainty was estimated as the root of sum of squares of the standard uncertainties of each component, according to Eq. 3. The results are shown in Table 6.

The reference value has the standard uncertainty that depends on the method that is used to derive it [19]. As the Botha et al. [2] mentioned, there is no general approach for the evaluation of uncertainty for all measurement procedures and materials. In our measurements, the uncertainty estimated for the reference value of the nitrogen mass fraction was 7.5% and does not differ from the uncertainty in the CRMs available on the market, e.g., uncertainty at the range of 7.7% for CRM

NCS DC73321; 8.7% for NCS ZC73001 or 9.4% for NCS DC73325 [6].

Conclusions

Based on the results of the evaluation of homogeneity and stability of the soil samples, it was found that the given material can be considered as the candidate for CRM. The use of two independent measurement procedures in terms of repeatability ensured the high level of the quality control. The presented procedures for evaluation of the homogeneity, stability and determination of the reference value with the uncertainty can be used for preparing laboratory reference materials (LRMs). Such LRMs can be used for routine quality assurance of soil samples, which is necessary to evaluate properties of the new fertilizers formulations. In this way, problem of unavailability of some matrix on the market of CRMs can be solved. It is economically justified as well.

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