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Standard operating procedure for soil organic carbon

Tyurin spectrophotometric method



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VERSION HISTORY

N°	Date	Description of the modification	Type of modification
01	13 January 2021	All comments by RESOLANs and reviewers to the draft SOP were addressed	Finalization of the SOP
02			
03			

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1. Brief introduction to soil organic matter (SOM)

Soil organic matter (SOM) is one of the most important components of soil formation, being a combination of specific and non-specific organic compounds. SOM is formed during the decomposition and humification of biogenic residues (Semenov and Kogut, 2015). The chemical structure of soil organic matter is complex and not fully known. Traditionally, the SOM content in soils is estimated by determining the concentration of carbon associated with organic compounds (C_{org}) and multiplying C_{org} by a factor of 1.724. The value of the coefficient was established from the condition that Carbon makes up nearly 58% of SOM. However, the carbon content of SOM may vary depending on the type of soil. Consideration of the possible variation in the organic matter composition predicts a range of factor values between 1.4 and 2.5 (Pribyl, 2010). Soil C_{org} is calculated by the stoichiometric reaction of carbon oxidation with potassium dichromate in concentrated sulfuric acid to carbon dioxide. This approach is implemented in Tyurin's method. The application of the Tyurin's method has historically been used in a large number of Eurasian countries.

2. Scope and field of application

This protocol applies to the determination of the oxidizable organic carbon content in the soil. The organic carbon content is calculated from the amount of chromic ion (Cr^{3+}) formed which is determined by the spectrophotometric method. The method is not suitable for soils with the presence of chloride Cl⁻ (> 0.6%), Fe²⁺, Mn²⁺. In these cases, Cr³⁺ is consumed for oxidation, and these interferences lead to an overestimation of the soil C_{org} content. This method is useful for soils with organic carbon content between 1.7 and 87 g kg⁻¹ (Metodika izmereniy N^o 88-17641-001-2020, Federal Information Fund for Ensuring the Uniformity of Measurements, Russia, FR.1.31.2020.38218, <u>https://fgis.gost.ru/fundmetrology/registry/16</u>). Errors in C_{org} determination are given in Table 1.

C _{org}		Trueness (limits of the systematic component of the relative error, $P = 0.95$)	Accuracy (limits of relative error, P = 0.95)
g kg⁻¹	%		
1.7-87 incl.	7.6	14	20

Table 1. Intra-laboratory precision, trueness and accuracy Corg measurement

3. Principle

Tyurin's method for measuring soil C_{org} is based on the oxidation of carbon by chromium in an acidic medium and the measurement of absorbance. The composition of SOM is different according to the type of soil, but the mean value of the degree of carbon oxidation in the soil is close to zero. The oxidation of organic carbon, which is part of the organic compounds of soils, by potassium dichromate in a sulfuric acid solution can be expressed by the equation:

$$3 C_{org} + 2 Cr_2O_7^{2-} + 16 H^+ = 3 CO_2 + 4 Cr^{3+} + 8 H_2O$$

The molar mass of carbon equivalents of organic compounds of the studied soils is taken to be $M(^{1}/_{4}C) = 3$ g mol⁻¹. In the system formed after oxidation, two components have absorbance in the visible region of the electromagnetic spectrum: dichromate anions at $\lambda < 575$ nm and complex chromium ions (III) with aqua-and sulfate-ligands (absorbance band maximum at $\lambda = 585 - 590$ nm (figures 1, 2)).

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The content of soil C_{org} is assessed by the absorbance of the complex chromium (III) compounds at a wavelength $\lambda = 590$ nm. In the absence of interferences, the number of equivalents of Cr³⁺ produced should be proportional to the equivalent amount of carbon of the organic compounds in the analyzed soils. The following reducing agents may be used to established the calibration of the spectrophotometers: Mohr's salt (iron (II) - ammonium sulfate hexahydrate) – Fe(NH₄)₂(SO₄)₂·6H₂O or sucrose – C₁₂H₂₂O₁₁. Both reducing agents provide the same measurement quality. The oxidation reaction of these reducing agents with the potassium dichromate in a sulfuric acid solution proceeds according to the equations:

$$Cr_2O_7^{2-}$$
 + 6 Fe²⁺ + 14 H⁺ = 2 Cr³⁺ + 6 Fe³⁺ + 7 H₂O

$$8 \operatorname{Cr}_2 \operatorname{O}_7^{2-} + \operatorname{C}_{12} \operatorname{H}_{22} \operatorname{O}_{11} + 64 \operatorname{H}^+ = 16 \operatorname{Cr}^{+3} + 12 \operatorname{CO}_2 + 43 \operatorname{H}_2 \operatorname{O}_2$$

When making measurements in the laboratory, the following conditions should be observed:

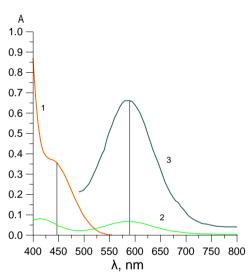
Α

from 15 °C to 27 °C

air temperature

atmosphere pressure from 84.0 to 106.7 kPa (from 630 to 800 mmHg.);

• relative air humidity not higher than 80%.



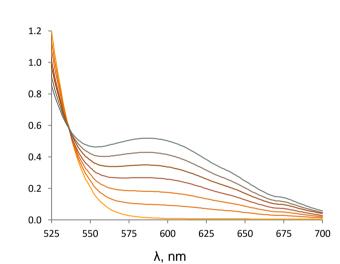


Figure 1 – Absorbance spectra of sulfate solutions containing dichromate anions or complex chromium cations (III): $c(^{1}/_{6}Cr_{2}O_{7}^{2-}) = 0.005 \text{ mol } L^{-1}(1), c(^{1}/_{3}Cr^{3+}) = 0.005 \text{ mol } L^{-1}(2), c(^{1}/_{3}Cr^{3+}) = 0.05 \text{ mol } L^{-1}(3)$

Figure 2 – Absorbance spectra of calibration solutions: $m(C_{org}) - 0, 1, 2, 3, 4, 5, 6$ mg per 25 mL of solution

Due to the large variation of procedures implemented by the GLOSOLAN laboratories using this method (Tyurin, 1931; Orlov, 1967; Simakov, Tsyplakov, 1969; Nikitin, 1983; GOST 26213-91; Samoilova, Rogiznaya, 2013), experts from the Institute of Biology Komi SC UrB RAS conducted a comparative study of reference and field soil samples by different variations of Tyurin's and Walkley-Black methods (Walkley, Black, 1934; Van Reeuwijk, 2002), using the element carbon analyzer EA-1110 (soil combustion in oxygen atmosphere at temperatures above 1000 °C). This research revealed that the incomplete oxidation of carbon in organic compounds by the Tyurin method can be taken into account by introducing a coefficient of 1.15. Nevertheless, this SOP ensures the comparability of the results obtained with the herewith described method and GLOSOLAN SOPs on Walkley-Black and Dry combustion. The results of this study are presented in Appendix I.

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Points to be noted:

- The efficiency of soil C_{org} oxidation ranges from 70 to 95 percent, depending on the type of soil, carbon speciation and the heating method, which can lead to a significant underestimation of the measurement results. In modern modifications of Tyurin's method, incomplete carbon oxidation of organic compounds in soils is not taken into account, although Tyurin's original work pointed out a coefficient of 1.17 (Arinushkina, 1962).
- This method is not recommended for soils with a significant content of carbonized materials.
- Saline samples may need specific preparation (Appendix II).

4. Apparatus

- Analytical balance with an accuracy of 0.0001 g
- Spectrophotometer suitable for measuring absorbance at a wavelength of 590 nm
- Centrifuge, maximum speed 6000 rpm
- Water bath
- Drying oven
- Bags made of polymer film materials
- Laboratory timer
- Metal spatulas
- Jasper mortars and pestles
- Pipettes 1 mL, 2 mL, 5 mL, 10 mL, 25 mL
- Dispenser 100 5000 μL
- Graduated cylinders 100 mL, 250 mL, 500 mL, 1000 mL
- Burette 25 mL
- Volumetric test tubes of 25 mL with an interchangeable cone of chemically resistant glass with ground stopper
- Centrifuge tubes, Conical
- Pasteur Pipette
- Laboratory funnel
- Tracing paper
- Aluminum tube rack
- Glass stick
- Porcelain mug
- Narrow-necked dark glass reagent flasks with ground glass stopper Conical flasks with ground glass stoppers 50 mL, 100 mL, 250 mL
- Chemical glasses 100 mL, 500 mL, 1000 mL
- Desiccator
- Platinum cup
- Evaporating cup
- Cup for weighing and storing substances
- Laboratory washer with screw cap and distribution tube

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5. Materials

- All chemicals should be of at least Analytical Grade.
- Deionized water/distilled water, it should have an EC < 0.001 mS cm⁻¹ (ASTM D1193-91 and ISO 3696:1987).
- Potassium dichromate solution (K₂Cr₂O₇) 0.136 M

Dissolve 20.0 g of potassium dichromate in 200 - 300 mL of distilled water in a 500 mL volumetric flask, then adjust to the final volume, stopper and thoroughly homogenized. The solution must be used during the day of its preparation.

• Chromium mixture – potassium dichromate in concentrated sulfuric acid

500 mL of concentrated sulfuric acid (not less than 96%) is added (in small portions) to an equal volume of 0.136 M potassium-dichromate solution. The solution is constantly stirred with 10 - 15 min breaks between each portion added and stored in a dark glass bottle for up to one year.

• Sulfuric acid solution (H₂SO₄) 0.5 M (1.0 N)

 V_a mL of sulfuric acid (Table 2) is added to 600 – 700 mL of deionized/distilled water, stirring constantly. When the solution has cooled down, it should be diluted to 1000 mL in a 1000 mL volumetric flask. The sulfuric acid solution is stored in a dark glass bottle for up to one year.

Density of solution at 20 °	Mass fraction of sulfuric acid	Molar concentration of sulfuric acid	Aliquot of the concentrated solution of sulfuric acid
ρ, g mL ⁻¹	ω(H ₂ SO ₄), %	<i>c</i> (H ₂ SO ₄), M	Va, mL
1.829	93.60	17.46	28.6
1.830	93.64	17.47	28.6
1.831	93.94	17.54	28.5
1.832	94.32	17.62	28.4
1.833	94.72	17.70	28.2
1.834	95.12	17.79	28.1
1.835	95.72	17.91	27.9

Table 2. Physico-chemical characteristics of a concentrated solution of sulfuric acid

• Potassium permanganate solution (KMnO₄) 0.02 M (0.1 N)

The solution of potassium permanganate is prepared from a commercially available standard solution (e.g. 109935 Supleco for 1000 mL Titrisol). The potassium permanganate is transferred into a 1000 mL volumetric flask and made to volume with deionized/distilled water. The solution is stored in a dark glass bottle with a ground stopper for up to six months.

• **Sucrose** 2.000 mg mL⁻¹

Weigh 1.1875 g of sucrose (dried at 105 \pm 2 °C for two hours, if stored in a desiccator, it must be used within 7 days) and dissolve in 250 mL deionized/distilled water in a 250 mL volumetric flask.

Mohr's salt (Ferrous Ammonium Sulphate) 0.2 M (0.2 N)

1) Dissolve 39.2 g of analytical grade $(NH_4)_2Fe(SO_4)_2.6H2O$ in 100 mL of sulfuric acid (0.5 M), and dilute it to a volume of 500 mL with distilled water.

2) The solution of Ferrous Ammonium Sulphate is prepared from a commercially available standard solution (e.g. 109864 Supleco for 250 mL Titrisol). The solution of 0.1 M $(NH_4)_2Fe(SO_4)_2$ of two ampoules is transferred to a 250 ml volumetric flask and brought to the volume with deionized/distilled water.

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Standardization of the reagent. A volume of 5.00 mL of the Mohr's salt solution is measured with a pipette in conical flasks (three replicates). Add to each flask: 1.0 mL of concentrated sulfuric acid (with a pipette) and 40.0 mL of distilled water (with a graduated cylinder). The obtained mixture is titrated with the solution of potassium permanganate until a faint pink colour appears that does not disappear for one minute. The concentration of iron (II) is calculated by the formula:

$$c(\frac{1}{1}\text{Fe}^{2+}) = \frac{c(\frac{1}{5}\text{MnO}_{4}^{-})\overline{V}(\text{MnO}_{4}^{-})}{V_{2}(\text{Fe}^{2+})}$$
(1)

Where $c(^{1/_1}Fe^{2+})$ - molar concentration of iron equivalents (II), N; $V_a(^{1/_1}Fe^{2+})$ - aliquot of the Mohr's salt solution taken for titration, mL; $c(^{1/_5}MnO_4^-)$ - molar concentration of potassium permanganate equivalents in the titrant, N; $\overline{V}(MnO_4^-)$ - average value of three volumes of the titrant, which was used for titration of an aliquot of the studied solution, mL.

The iron (II) solution is not usable when $c(^{1}/_{1}Fe^{2+}) = 0.195$ N.

The solution is stored in a dark glass bottle for up to six months. The molar concentration of iron equivalents (II) is checked by titration with a potassium permanganate solution (in case of prolonged interruptions in the use of the solution, the titration should be done before graduating the spectrophotometer).

6. Health and safety

This procedure involves the use of hazardous chemicals. Refer to the laboratory safety guidelines.

6.1. Personnel safety

Safety glasses, gloves and lab coats must be worn when handling any chemical.

6.2. Chemical hazard

- 1. Potassium dichromate is an inorganic compound that emits toxic chromium fumes upon heating. Potassium dichromate is highly corrosive and is a strong oxidizing agent. This substance is a known human carcinogen and is associated with an increased risk of developing lung cancer.
- 2. Sulfuric acid should be kept away from heat and open flames. The concentration should be measured in air regularly. Operations should be carried out in a fume hood with exhaust/ventilation. Waste should not be discharged into the drain. Sulfuric acid should never be diluted by pouring water into it. The acid should be added to the water.
- 3. Hygiene: it is necessary to wash hands and clean other exposed areas with mild soap and water after using all chemical reagents.

6.3. Soil sampling and storage

The sampling and storage of soil samples is carried out under conditions that ensure the safety of the sample composition.

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7. Sample preparation

Soil samples are prepared under conditions that ensure the composition of the sample in accordance with the regulatory and technical documentation for the objects studied. Air-dry samples of soils are obtained by drying at the temperature and humidity of the laboratory room. A portion of the soil sample is taken and scattered on tracing paper, large inclusions (undecomposed roots and plant debris, stones, tumors, etc.) are removed with tweezers.

The soil sample is sieved (< 1 mm or other diameter, according to the procedures of each country) and one portion is grinded in a jasper or agate mortar to a size of 0.25 mm in diameter. The prepared soil samples are stored in plastic bags, plastic or glass containers. The mass of the air-dry soil sample to be analyzed shall be taken on the basis of the estimated organic carbon content according to Table 3, which can first be judged by the colour of the soil or, if known, by its type.

Soil colour	C _{org} SOM		Weight of soil sample
			m
	g kg ⁻¹		mg
Whitish	From 1.7 to 6 incl.	From 3 to 10 incl.	From 500 to 900
Light grey, light brown	From 6 to 12 incl.	From 10 to 20 incl.	From 200 to 500
Grey, brown	From 12 to 30 incl.	From 20 to 50 incl.	From 100 to 200
Dark grey, brown	From 30 to 60 incl.	From 50 to 100 incl.	From 60 to 100
Dark brown, black	From 60 to 87 incl.	From 100 to 150 incl.	From 30 to 60

Table 3. Recommended weighed samples of soils (Kotova et al., 2007)

8. Procedure

8.1. Preparation of standards curve

To prepare calibration solutions, a standard titre of Mohr's salt or sucrose is used.

Attention! Tubes containing blank solutions (chromium mixture only) and calibration solutions (chromium mixture with sucrose), should be prepared by heating in a boiling water bath simultaneously with tubes containing mixtures of soil working samples and samples for the inter-laboratory quality control of the measurement results with an oxidizing agent.

In this regard, it is necessary to choose a tube rack with a sufficient number of sockets for test tubes with simultaneously heated systems. Glass rods are inserted into the test tubes with systems prepared for heating, the systems are mixed and transferred to a tube rack immersed in a boiling water bath. The water level in the bath should be at least 2 cm higher than the level of the chromium mixture in the test tubes. A water bath with test tubes is heated for 1 hour from the moment the water boils in the bath. During heating, the systems in the test tubes are periodically (at least twice) mixed with glass rods. The tubes are removed from the water bath, placed in a tube rack and cooled by immersion in a container of cold water.

8.1.1. Preparation of a set of sucrose standards (0-6 mg C) in tubes

In seven labelled 25 mL tubes, add 10 mL of the chromium mixture and the appropriate volume of the sucrose standard (according to the Table 4). Heat the solutions in a water bath. After cooling the chromium

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mixture with sucrose, the glass rod is successively lifted from the solution and, while holding it inside the tube, washed with 10 mL distilled water ($V_1(H_2O)$ in Table 4), measured with a pipette or a dispenser. The rod is removed from the tube. Additional distilled water is added ($V_2(H_2O)$ in Table 4).

Table 4. The composition of sucrose standards

Mass of C _{opr} , mg		0	1.00	2.00	3.00	4.00	5.00	6.00
Chromium mix volume, mL		10.0	10.0	10.0	10.0	10.0	10.0	10.0
Sucrose standard (2 mg Corg	mL ⁻¹), mL	0	0.50	1.00	1.50	2.00	2.50	3.00
Volume of distilled water	V ₁₍ H2O), mL	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Volume of distilled water	V ₂₍ H2O), mL	5.00	4.50	4.00	3.50	3.00	2.50	2.00

8.1.2. Preparation of a set of Mohr's salt standards (0-6 mg C) as specified in the table below in tubes

10.0 mL of chromium mixture is poured into each of the seven labelled test tubes. The tubes are heated in a water bath. The solutions are cooled. Holding glass rods inside the test tube, wash with distilled water. The glass rods are taken out. The Mohr's salt solution is added. The composition of Mohr's salt standards is presented in Table 5.

Table 5. The composition of Mohr's salt standards

Equivalent mass of Copr, mg	0	0.60	1.20	2.40	3.60	4.80	6.00
Chromium mix volume, mL	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Volume of distilled water, mL	15.0	14.0	13.0	11.0	9.0	7.0	5.0
Mohr's salt Standard ($c(^{1}/_{1}F_{1}Fe(NH_{4})_{2}(SO_{4})_{2}))=0.200 \text{ mol } L^{-1}), \text{ mL}$	0	1.00	2.00	4.00	6.00	8.00	10.00

The tubes are closed with stoppers, stirred by turning each tube 3-5 times, and kept for 24 hours in a fume hood at room temperature and protected from light, together with the mixtures of the studied soils and the quality control samples with the oxidizing agent, according to 8.3.

8.2. Measuring the absorbance of calibration solutions and estimating the coefficients of the calibration function

The absorbance of the calibration solutions sustained during the day is measured at a wavelength of 590 nm in cuvettes with an absorbing layer thickness of 1.00 cm relative to the reference system – distilled water. The least squares method is used to calculate the values of the coefficients of the calibration function:

$$A = A_{\rm o} + Km(C_{\rm org}).$$
⁽²⁾

Where A – absorbance of calibration solutions at λ = 590 nm;

- *m*(C_{org}) the mass of carbon of organic compounds in calibration solutions (in an aliquot of the CM taken to prepare the calibration solution), mg;
- A_o calculated absorbance of blank calibration solution.

The value of the correlation coefficient (*R*) of the calibration function must be \geq 0.9995.

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8.3. Preparation of soils samples for measurements

One weighed portion of each soil sample and CS are placed in test tubes with a capacity of at least 25 mL and placed in racks. A volume of 10.0 mL of the chromium mixture is added to each tube using a measuring pipette and the obtained mixtures are heated in a water bath. After cooling the mixtures, the glass rod is subsequently lifted from the mixture and, holding it inside the tube, washed with 15 mL of distilled water measured with a pipette or dispenser.

The tubes are closed with stoppers, stirred by turning each tube 3-5 times, and left to allow the solid particles to settle for 24 h in a fume hood at room temperature, protected from light. After partial or complete settling of the mixtures, a portion of 7 to 10 mL of the suspension (or solution) is decanted into centrifuge tubes. To balance the masses of the tubes, an adjustable pipette is used to collect a constant volume of the supernatant suspension (or solution). A clean and dry tip is used for each tube.

The prepared tubes are centrifuged until a clear solution is obtained. The centrifugation time is selected experimentally depending on the number of rpm. For example, at 6000 rpm, the required centrifugation time is 5 – 10 minutes.

Note - If, after one day, the supernatant is visually transparent, centrifugation is not needed and it is admissible to transfer it directly, and carefully, into the cuvette.

The absorbance of the samples is read from a spectrophotometer set at a wavelength of 590 nm.

In a test batch of soils, a blank solution is used every ten solutions. In calculation formula 3, the average absorbance of all blank solutions (A_{blank}), including A_0 (formula 2), is used.

9. Calculation

The Corg content of the unknown samples is calculated according to:

$$C_{\text{org}} = \frac{f(A-A_{\text{blank}})}{Km} \mathbf{1000} \quad (g \text{ kg}^{-1})$$
 (3)

Where

Α

absorbance of study solution; _

Ablank - absorbance of blank; Κ coefficient of calibration function, mg⁻¹;

- sample weight of the test soil, mg.

М f = 1.15 – correction factor taking into account the incomplete oxidation of carbon of organic compounds by the Tyurin method.

The mass fraction of organic matter is calculated by the formula:

OM (g kg⁻¹) = 1.724
$$C_{org}(g kg^{-1})$$
. (4)

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10. Quality assurance/quality control

The frequency of obtaining the results of control procedures and the forms of their registration are given in the laboratory documents, which shall establish the procedure and content for the organization of methods for monitoring the quality and stability of the measurement results in the laboratory.

10.1. Operational control of the intra-laboratory error of the measurement results using quality control samples

The matrix of soils used for quality control of soil samples with the certified value of C_{org} or OM content should be within the range of the soils under study.

When selecting quality control samples, it is necessary to take into account the method of measurement used to obtain the certified value of the mass fraction of carbon of C_{org} or OM.

- 1. If the certified value of C_{org} or OM content is obtained under conditions of complete oxidation of organic compounds (dry combustion method), the measurement results calculated by equation (4), f = 1, are compared with the certified value of CS.
- 2. If the certified value of C_{org} or OM content is obtained by the Walkley-Black method, the measurement results calculated by equation (4), f = 1.3, are compared with the certified value of CS.
- 3. If the certified value of C_{org} or OM content is obtained by the Tyurin's method (for example, GOST 26213), the measurement results calculated by equation (4), f = 1.15 are compared with the certified value of CS.

To control the accuracy of the measurement results of C_{org} content, C_{org} content in the CS is measured. The result of the control procedure (K_1 , %) is calculated by the formula:

$$K_1 = \frac{|C_{\text{org}} - \dot{C}_{\text{org}}|}{\dot{C}_{\text{org}}} 100.$$
(5)

Where C_{org} – measured value of the mass fraction of carbon of organic compounds in the CS, g kg⁻¹; C'_{org} – certified value of the mass fraction of carbon of organic compounds in the CS, g kg⁻¹.

To control the accuracy of the measurement results of the mass fraction of organic matter, the mass fraction of organic matter in the CS is measured:

$$K_1 = \frac{|OM-OM'|}{OM} 100.$$
 (6)

The relative discrepancy between the measurement result and that given in the certificate of the standard should not exceed 20% (Table 1). If the condition is not fulfilled, the control procedure is repeated. If this condition is not fulfilled again, the reasons for unsatisfactory results must be found and measures taken to eliminate them. With a satisfactory control of the error, the accuracy of the measurement results of the entire batch of soil samples is considered satisfactory.

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10.2. Operational control of the intra-laboratory error of the measurement results using another (control) method

For control purposes, a technique, in which complete oxidation of organic compounds is carried out (for example, Dry combustion method), is used. CS is taken from a batch of test soils. Corg is measured in the CS by two methods – Tyurin and Dry combustion.

The relative discrepancy between the measured value of the carbon mass fraction by the Tyurin's method C_{org} and Dry combustion C'_{org} is calculated according to the formula:

$$K_2 = \frac{|\mathsf{C}_{\mathsf{org}} \cdot \dot{\mathsf{C}}_{\mathsf{org}}|}{\mathsf{C}_{\mathsf{org}}} 100.$$
 (7)

The result (K_2 ,%) should not exceed the standard for monitoring the accuracy of the measurement results of the Dry combustion method. If the condition is not fulfilled, the control procedure is repeated. If this condition is not fulfilled again, it is necessary to find the reasons for unsatisfactory results, and take measures to eliminate them. With a satisfactory control of the error, the accuracy of the measurement results of the entire batch of soil samples is considered satisfactory.

Frequency of error control of measurement results. Control of the internal laboratory error of the measurement results of C_{org} or OM is carried out according to the schedule established in the testing laboratory.

10.3. Algorithm for controlling intra-laboratory precision

The conclusion on the acceptability of the measurements results of C_{org} or OM in soil samples is made according to the results of the laboratory precision control. Intra-laboratory precision control of the measurement results of C_{org} or OM is carried out using working samples of soils that are selected from a batch of test samples, according to the schedule established in the laboratory (for example, every tenth).

Corg is measured twice under reproducible conditions: primary (Corg) and repeated results (Corg).

$$R = \frac{|\mathbf{C}_{\rm org} - \mathbf{C}_{\rm org}|}{\overline{C_{\rm org}}} \tag{8}$$

$$\overline{C_{\text{org}}} = \frac{C_{\text{org1}} + C_{\text{org2}}}{2}.$$
(9)

Where $\overline{C_{org}}$ – arithmetic mean of two measurement results of the mass fraction of carbon of organic compounds in the sample for control, g kg⁻¹;

The relative discrepancy between the two measurements should not exceed 21%. If the result of the intralaboratory precision control of C_{org} or OM is satisfactory, the measurement results in the entire batch of samples obtained in a single measurement are acceptable. Both measurement results are acceptable for CS of soils, and their arithmetic mean value can be used. If the condition is not fulfilled, additional methods can be used to verify the acceptability of the measurement results.

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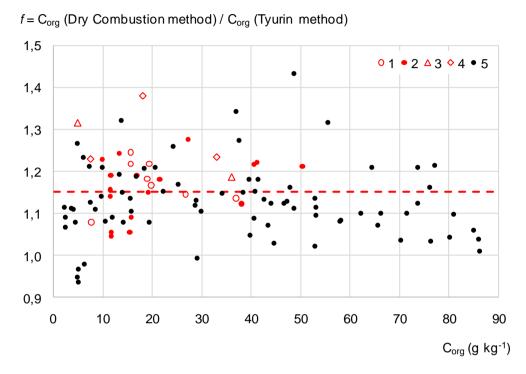
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Appendix I – Estimation of the coefficient taking into account incomplete oxidation of carbon in organic compounds in the Tyurin method





1 - Reference materials of soils (Elemental Microanalysis Limited, UK);

2, 3 - Quality control soil samples (Russia);

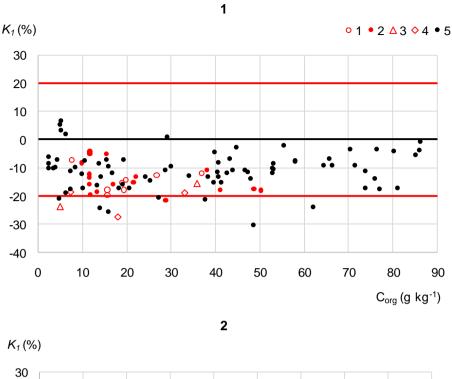
4 – Quality control soil samples (GLOSOLAN);

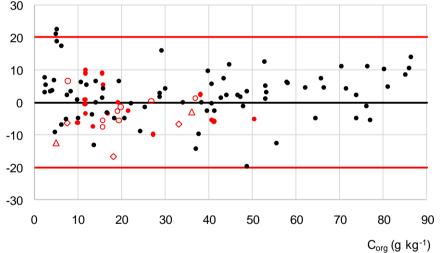
5 - Soil samples of various types, more than 100 soil samples in total.

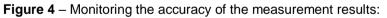
Mean f = 1.15.

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- 1. oxidized by dichromate anions in a sulfuric acid solution without taking into account the coefficient f = 1.15;
- 2. taking into account the coefficient of f = 1.15 (taking into account the incomplete oxidation of carbon of organic compounds under the conditions of the Tyurin's method).

A comparative study of quality control samples and field soil samples by Tyurin, Walkley-Black and Dry combustion methods confirmed the possibility of taking into account incomplete organic carbon oxidation. For this, it is necessary to enter a coefficient of 1.15 for the Tyurin's method and 1.3 for the Walkley-Black method (additional information are available <u>here</u>).

Walkley-Black \cdot 1.3 = Tyurin \cdot 1.15 = Dry Combustion (*P* = 0.95).

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Appendix II - Preparation of saline samples

A weighed portion of the test soil from 5.00 to 10.00 g (m_1 , g) is placed in a beaker and 100 or 200 mL (20-fold volume) of distilled water and 1 mL of sulfuric acid solution ($c(^{1}/_{2} H_{2}SO_{4}) = 1 \text{ mol } L^{-1}$) is added. The sample is periodically mixed during the working day and left until the next day. The transparent solution is drained by decantation, without losing or stirring up the precipitate (if the precipitate is still suspended, 1ml of sulfuric acid solution is added, mixed and left until the solution becomes clear). The precipitate is poured with a second portion of distilled water, acidified with a solution of sulfuric acid, mixed and left to precipitate the solid phase. Using a pipette, 0.5 - 2 mL of a clear solution is carefully selected into a test tube and 2 – 5 drops of silver salt solution (AgNO₃ = 10 g kg-1 or Ag₂SO₄ = 10 g kg-1)1 are added. In case of precipitation of silver chloride, the washing procedure of the test material is continued until there are no chloride ions in the supernatant.

The clear solution is drained by decantation. A volume of 5 - 10 mL of distilled water is added to the precipitate that does not contain chloride ions, the mixture is shaken and transferred to a cup (porcelain or platinum), previously weighed (m_0 , g). Portions of distilled water are poured into a glass until all the sediment is transferred to the cup. The cup with a mixture of a sample soil and distilled water is placed in a water bath and the water is evaporated until dry soil remains in the cup.

The cup with the soil is left at room temperature for a day to establish an equilibrium between soil moisture and air (air-dry state). A chloride-ions-free air-dry sample is weighed into (B) a cup (m2, g). The mass fraction of carbon of organic compounds in an air-dry soil sample that does not contain chloride ions (C_{org} ', g kg⁻¹), calculated by formula 5), is measured by performing all the procedures and correcting the weight loss during soil washing:

$$C_{org} = \frac{m_2 - m_1}{m_1} C'_{org}$$
 (10)

¹ A portion of 0.5 g of silver salt is dissolved in 50 mL of distilled water. A portion of silver salt is permissible to change while maintaining the ratio of the mass of salt and the volume of distilled water.

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Appendix III - Acknowledgements

GLOSOLAN would like to thank Ms. Elena Shamrikova and colleagues at the Eco-analytical laboratory of the Institute of Biology of Komi Scientific Center of the Ural Branch of the Russian Academy of Sciences, Russian Federation for leading the harmonization of this SOP. The study was carried out in the framework of the RFBR grant No. 20-04-00445a "Factors and mechanisms of stabilization of organic matter in soils of extreme Environment (on the example of Arctic ecosystems)". GLOSOLAN would also like to thank the experts who were part of the Review Panel and who ensured the finalization of the SOP, and all laboratories that provided inputs for the harmonization of this method, all the experts who technically reviewed this SOP and Mr. Rob De Hayr from the Chemistry Centre Laboratory - Queensland Department of Environment and Science, Australia for the technical proofreading of the document.

Appendix IV - List of authors

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Appendix V - Contributing laboratories

GLOSOLAN would like to thank the following laboratories for completing the GLOSOLAN form on the method and providing information on their standard operating procedure for the Tyurin spectrophotometric method, which were used as a reference for doing the global harmonization:

From the Asian region:

- Soil and Agrochemistry laboratory, school of Agroecology, Mongolian University of Life Sciences, Mongolia
- Soil, Agro-chemistry laboratory of the Institute of Plant and Agricultural Sciences, Mongolia
- Laboratory of Soil Science, Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, **Thailand**

From the Pacific region:

None

From the African region:

- Centre d'etude et de Recherche de Djibouti, Laboratoire de Pedologie, Djibouti
- Laboratoire Central de l'Institut de Recherche Agronomique de Guinée, Guinée

From the Near East and North African region:

• National Agricultural Research Center, Jordan

From the European region:

- Forest Research Institute, Bulgaria
- University of Zagreb, Faculty of Agriculture, Soil Science Department, Croatia
- University of Zagreb, Faculty of Agriculture, Department of General Agronomy, Croatia
- Central Institute for Supervising and Testing in Agriculture, Czechia
- State Plant Protection Service, Agrochemical Laboratory, Latvia
- University "St. Kliment Ohridski"- Bitola Scientific Tobacco Institute Prilep, Republic of North Macedonia

From the Eurasian region:

- Laboratory of Chemical and Biological Factors, Ukraine
- Lomonosov Moscow State University, Russian Federation

From the Latin American region:

- Ministry of Agriculture, Haiti
- Colegio de Postgraduados, México

From the North American region:

• None

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GLOSOLAN GLOBAL SOIL LABORATORY NETWORK

GLOSOLAN is a Global Soil Laboratory Network which aims to harmonize soil analysis methods and data so that soil information is comparable and interpretable across laboratories, countries and regions. Established in 2017, it facilitates networking and capacity development through cooperation and information sharing between soil laboratories with different levels of experience. Joining GLOSOLAN is a unique opportunity to invest in quality soil laboratory data for a sustainable and food secure world.

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