Food and Agriculture Organization of the United Nations

Standard operating procedure for quasi total elements in soil by acid digestion, including heavy metals

GLOSOLAN

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QUASI TOTAL ELEMENTS IN SOIL BY ACID DIGESTION, INCLUDING HEAVY METALS

VERSION HISTORY

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GLOSOLAN SOP Tech. W.G. Global leaders: Laura Marcela Casas Cevallos, Colombia Marie-Pierre Tine, Senegal	By GLOSOLAN Technical Committee and INSOP experts	16 January 2023	16 January 2023

Contents

1. Introduction

There are around 80 constituents of earth's crust, known as trace elements (TE) found at concentrations less than 0.01 percent in soil. They make up around 0.6 percent of all the elements by mass, while the 12 major elements (oxygen (O), silicon (Si), aluminum (Al), iron (Fe), calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), titanium (Ti), hydrogen (H), phosphorus (P) and manganese (Mn)) make up the other 99.4 percent. Some of these trace elements are metals (MTE) such as: cadmium (Cd), chromium (Cr), zinc (Zn), lead (Pb), cupper (Cu) and others are metalloids for example arsenic (As), antimony (Sb), selenium (Se) and boron (B).

Some TE are vital for biological processes, therefore they are vital for plant and animal growth, such as B, Cu, Fe, Mn, molybdenum (Mo), nickel (Ni) and Zn. Others are non-essential like Cd, Pb, mercury (Hg) and tin (Sn). Some of them, are potentially toxic pollutants depending on their concentration as Zn, Cr, Cd, Pb, Hg and Sn, but most of all, depending on their chemical form (speciation and solubility). These are often known as "heavy metals" which is the most popularly used and widely recognized term for a large groups of elements with density greater than 6 g/cm. However, not all TEs are metals.

In order to determine the true value of total trace elements in soil, an analytical process is required. That should aim to extract and solubilize all the element forms to be analyzed, including those bound into the crystal lattices of silicate minerals. The only way to get the latter into solution is to use dangerous and toxic hydrofluoric acid to dissolve the silica. However, a good close estimate of this true total (or quasi-total) can be obtained using the so-called "partial" dissolution by aqua regia (hydrochloric acid (HCl) + nitric acid (HNO_3)) with a heating system such as closed microwave, heating block or under boiling in a glass tube.

The resulting solution is suitable for the determination of trace elements using atomic absorption spectrometry (AAS), inductively coupled plasma - optical emission spectrometry (ICP-OES), microwave plasma-atomic emission spectrometer (MP-AES) or inductively coupled plasma - mass spectrometry (ICP-MS).

2. Scope and field of application

This method is applicable to the determination of MTE trace elements such as P, Ca, Mg, K, Na, Cd, Pb, Cr, Ni, Cu, Fe, Mn, Zn, Co, Mo, Al, silver (Ag), Sb, barium (Ba), Hg as well as metalloids like As, Se in soils by the aqua regia (HCl + HNO_3 in a 1:3 ratio).

Regarding Hg determination, during sample digestion special care must be taken with the seal of the microwave digestion bomb, otherwise this metal may be partially volatilized. Considering that, traditional hot plate method is not recommended for Hg determination.

This method is based upon the US EPA method 3051 (Element, 2007) and the one proposed by Rayment and Lyons (2011). This method covers the determination for all types of soils. However, when soil samples with high carbon content (more than 20 percent oxidizable carbon) are analyzed, the US EPA 3050B

method (Edgell, 1996) using repeated additions of concentrated HNO_3 and 30 percent hydrogen peroxide (H_2O_2) may be preferred. Both methods employ heating by closed microwave or open heating system. Before carrying out the determination, it is necessary that the sample to be analyzed has undergone a pre-treatment, which corresponds to a previous drying at room temperature or in an oven below 40 °C, and a particle size reduction by fine grinding, such as in a ring pulverizer.

3. Principle

The procedure described in this document refers to two methods of digestion:

- **Extraction method with a microwave digester or a heating block**

  The sample is subjected to the action of aqua regia (mixture of HCl and HNO_3) overnight at room temperature, then boiled for two to five hours. The extract is then filtered and adjusted to volume with demineralized water.

- **Extraction method with a microwave oven in a closed reactor**

  The sample is subjected to the action of aqua regia (mixture of HCl and HNO_3) by microwave according to a defined program. The extract is then filtered and adjusted to volume with demineralized water.

In both methods, digestion is performed by acid addition and heating. The extract contains most of the trace elements that were in the soil as solution, and their concentrations can then be determined using spectrometric methods.

4. Apparatus

- analytical balance, precision ± 0.0001 g
- micro spatula
- mechanical pipette and tips or glass pipette
- 100 mL beakers or 50 mL plastic bottles
- watch glasses suitable for beakers or plastic bottles
- wash-bottle
- filter paper, ashless
- filter funnels (made of glass, polypropylene or other interference free)
- volumetric flasks of 100 mL or 50 mL
- beaker 100 mL, 50 mL
- oven able to reach a temperature of 105 °C
- microwave heating system (175 °C to 200 °C)
- heating block or mineralizer (95 °C to 105 °C)
- fume hood extraction/ventilation
- atomic absorption/emission spectrophotometer suitable for measuring absorbance at 627 nm wavelength, either AAS, MP-AES, ICP-OES or ICP-MS

5. Materials

- Deionized water/distilled water, It should have a specific conductivity not higher than 0.2 mS/m at 25 °C and a pH greater than 5.6 (or grade 2 water or type II water according to ISO 3696 (1995) and ASTM D1193-06 (2018), respectively, if this is the quality of water produced in the laboratory);
- Concentrated nitric acid (HNO_3), 15,8 mol/l – 1,42 g/ml;
- Concentrated hydrochloric acid (HCl), 12,0 mol/l – 1,19 g/ml;
- 5% V/V hydrochloric acid: Pour 50 mL of concentrated HCl into 500 mL of ultra-pure water; leave to cool and gauge to 1 liter;
- 2% V/V nitric acid: Pour 20 mL of concentrated HNO_3 into 500 mL of ultrapure water; leave to cool and gauge to 1 liter.

Acids should be tested for freedom from interferences. Alternatively a blank digestion is performed for correction of final results.

6. Health and safety

Each chemical compound used in the procedure must be treated as presenting a potential health hazard, meaning to keep exposure time to the compounds to a minimum. Refer to the specific material safety data sheet for hazardous properties of any chemicals or reagents used in this assay, before proceeding.

Digestions with hydrochloric acid and nitric acid are potentially dangerous. Wear safety glasses, gloves and lab coats when performing this analysis. Digestion should be carried out under a properly ventilated fume hood. Wearing a mask is mandatory during acid handling. The use of strongly oxidizing reagents can lead to the formation of explosive organic derivatives. Do not open pressurized containers until they have cooled.

The wearing of a mask particularly during sample preparation should also be encouraged and the same on sample disposal and washing up.

Check the correct handling and operation instructions of the equipment involved before use.

Chemical hazards

- Nitric acid (65-70%) is considered hazardous by OSHA (2012). It may cause fire or explosion, and it is a strong oxidizer. The use of strongly oxidizing reagents can lead to the formation of explosive organic derivatives, especially for samples with very high organic content. It may be corrosive to metals and cause severe skin burns, eye damage, and respiratory irritation.
- Hydrochloric acid causes severe skin burns and eye damage. It may cause respiratory irritation. Do not discharge the waste into the drain.
- Ammonium molybdate solution is a corrosive liquid. Contact with the eyes or body can cause serious health hazard. Reaction with metals may produce hydrogen gas and oxides of sulphur may be produced in a fire.

- Potassium antimony tartrate is hazardous in case of skin/body contact. Do not discharge the waste into the drain. It is incompatible with strong acids, strong bases and strong oxidizers. Do not expose to direct sunlight.
- Ascorbic acid has no known effect on the skin/body but should be stored in light-resistant containers because it is light sensitive. Keep away from incompatibles materials, such as oxidizing agents.

Wash hands and clean other exposed areas with mild soap and water after using all chemical reagents. Always remember to not open pressurized containers until they have cooled. Never dilute by pouring water into the acid, always add the acid to the water.

7. Sample preparation

It is necessary to grind the sample finely for this analysis. In order to do that, the sample needs to be air-dried first. Results should be reported on an air-dry basis, or mentioning the soil moisture content measured on an oven-dry basis.

The pretreatment consists of air drying the soil, or in an oven at a temperature below 40 °C. Then, particle size is reduced to 0.2 mm (70 mesh) by milling, for example in a ring pulveriser. Ensure that the grinding equipment and all materials used do not introduce sample contamination.

Finally the pretreated sample must be stored in an airtight polyethylene container, duly identified.

The appropriate storage conditions for the samples is at a temperature between 3 °C and 30 °C, free from direct sunlight.

For further details regarding this part of the procedure, refer to the GLOSOLAN SOP on handling and preparation of soil samples for chemical and physical analyses.

8. Procedure

8.1. Extraction method by open digestion system (hot plate or mineralizer block)

8.1.1 **Cold digestion**

8.1.1.1 Weigh very precisely between 0.5 g and 1 g of ground soil in a 100 mL beaker or 50 mL plastic bottle. Record the weight;

8.1.1.2 Add 7.5 to 10 mL of 37% HCl and 2.5 to 5 mL of 65% HNO_3;

8.1.1.3 Allow to stand without heating overnight.

8.1.2 **Hot Digestion**

8.1.2.1 Boil the previous solution for 2 to 5 hours (95 to 105 °C) covering it with a watch glass;

8.1.2.2 Remove the watch glass and dry;

8.1.2.3 Add 25 mL of 5% HCl, maintain the heating for about 10 minutes, then cool at room temperature. Filter and adjust very precisely to 50 mL or 100 mL with demineralized water in a volumetric flask.

8.2. Extraction method by closed digestion system (microwave)

8.2.1 Weigh about 0.5 g and 1 g of ground soil and transfer it to the reactor. Record the weight.

8.2.2 Add 3 mL of HCl (32 to 37%) and 9 mL of HNO_3 (65 to 77%) drop by drop if necessary to reduce the formation of foam.

8.2.3 If a strong reaction occurs (generation of bubbles), allow the reaction to subside before closing the container. Allow to stand overnight without heating. Transfer the digestion vessels to the cavity of the microwave unit and start the digestion mode according to the manufacturer's instructions (10 to 30 min, temperature 175 °C to 200 °C).

8.2.4 At the end of the program, allow to cool to room temperature to reduce the pressure in the container. Carefully filter the supernatant containing relatively little suspended solids through filter paper, collecting the filtrate in a 50 mL or 100 mL volumetric flask.

8.2.5 Allow all the initial filtrate to pass through the filter paper, then wash the insoluble residue on the filter paper with demineralized water and adjust to 50 mL or 100 mL with demineralized water. Final volume can be 50 mL or 100 mL according to the volumetric flask used, but this volume must be taken in account during the calculations.

The sample is now ready for analysis by Atomic Absorption Spectrometry (AAS) or Atomic Emission Spectrometry (AES) or Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) or Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Methods were listed in order of increasing sensitivity and decreasing limit of detection.

Analysis of Hg with ICP-MS is poor due to the memory effects on this type of equipment.

8.3. Quantify the elements with MP AES, ICP OES or AAS:

8.3.1 Set up the instruments according to manufacturer’s instructions and internal standard operating procedures.

8.3.2 Prepare the calibration curve taking in account the expected contents in the samples. Make the dilutions if necessary or resume with a more suitable range of standard points.

8.3.3 Standard points and dilutions are carried out with a 2% HNO_3 solution. Prepare the range with a multi-element standard solution with 7 points ranging from 0 to 20 mg/l and complete with a 2% nitric acid solution.

The curve range can be modified according to the quantification equipment.

9. Calculation

The amount of each element is calculated and expressed as mg/kg as shown in equation 1:

$$\boldsymbol{C} = \frac{\left((\boldsymbol{R_s} \times \boldsymbol{D}) - \boldsymbol{Bl}\right) \times \boldsymbol{V} \times \boldsymbol{Fh}}{\boldsymbol{M_s}} \qquad (\mathbf{1})$$

Where:

C = concentration in mg/kg

R_s = spectrometer result, in mg/L

D = dilution factor, if any

Bl = concentration of method blank (see section 10.4)

V = final volume (50 mL or 100 mL)

Fh = moisture content correction factor (see Appendix I)

M_s = mass of soil, in g

The result must be reported with 2 decimal places.

Note: subtraction of method blank at equation (1) is not recommended for analysis with ICP-OES. Instead, control over blank method values should be implemented.

10. Quality assurance / quality control

10.1. Accuracy test

10.1.1 Analysis of CRM (NIST traceable or other) should be performed to ensure quality results (about every 20 samples and to verify the prepared calibration curve. A concentration of about the middle of the calibration curve can be verified (or different levels of concentration), and the error should be calculated as:

$$\%\,Error = \left|\frac{C_{\,Reported\ value} - C_{\,True\ value}}{C_{\,True\ value}}\right| \times 100\%$$

Where C is the concentration in mg/L

10.1.2 Participation in Inter-laboratory Proficiency Test (PT) at least once a year. The PT z-score should be less than 2. If not, identify root cause, develop corrective and preventive actions.

10.2. Precision test

Perform replicate analysis (duplicate) every 10-20 sample in each batch test. The RPD should not be greater than 10 percent between results.

$$RPD = \frac{M_1 - M_2}{\left(\frac{M_1 + M_2}{2}\right)} \times 100\%$$

Where:

M_1 = result of sample

M_2 = result of sample's duplicate

RPD = relative percent difference

10.3. Laboratory control sample

The measurement of an in-house or purchased reference sample can be analyzed per batch of analysis, for ensuring normal conditions on the process and materials. This laboratory control sample can be known as the internal reference sample or master sample. Its use can include a control chart of RPD values (when the control sample is analyzed per duplicate). It should be available in sufficient amount to act as an internal control or reference for a reasonable length of time ideally over 12 months.

10.4. Method Blank

A method blank, using an empty digestion vessel (as the same used for other soil samples), is carried through all the steps of the procedure (digestion, filtering, dilution), for tracing any contamination in the material and in the reagents. Blank analysis every 15 or 20 samples should be done, ensuring blank results are lower than the Low Limit of Quantitation (LOQ) for each element.
It is recommended to have statistical data for blank results over time, avoiding errors when subtracting the method blank concentration to results.

10.5. LOD and LOQ

Limit of detection (LOD) is the approximate concentration that causes difficulty detecting the analyte. It can be estimated by analyzing at least 10 replicates of blank samples or reagent blanks as:

$$LOD = 3 \times s'$$

$$1)\ s' = \frac{s}{\sqrt{n}} \quad or \quad 2)\ s' = s\sqrt{\frac{1}{n} + \frac{1}{n_b}}$$

Where:
s' = standard deviation for LOD and LOQ calculation, calculated by 1) if results were obtained without blank correction, or 2) if blank corrections where applied
s = standard deviation of the results of blank replicates
n = Number of replicate observations averaged (each replicate obtained following the entire measurement procedure)
n_b= number of blank observations averaged for blank correction (according measurement procedure)

Limit of quantitation (LOQ) is the lowest concentration that can be determined with acceptable performance. It can be obtained by analyzing at least 10 replicates of blank samples or reagent blanks spiked at low concentrations, as:

$$LOQ = k \times s$$

Where:
k can be 10 or 5 based on fitness purpose criteria (see reference 9)
s' = standard deviation calculated as described above

The LOD and LOQ should be stablished for each analyte on the applied methodology.

10.6 Control charts

For this type of analysis, several types of control charts could be used:

- A chart for registering RPD values of each batch test. The chart should include the limit RPD value stablished by the laboratory.
- A chart for the error percentage for each element during the CRM analysis can be plotted, establishing a limit range of error (10 percent to 20 percent depending on concentration).
- Sometimes, a chart of method blank values can also be used, establishing maximum blank values accepted.

10.7. Spiking recovery

Spiking the digested extract, could help to analyze possible matrix interferences on the quantification of the sample. The spiking volume should be less than two percent of the sample's volume, and recovery percentage should be calculated as:

$$\%\,Recovery = \frac{C_{spiked} - C_i}{C_{added}} \times 100\%$$

Where:
C_{spiked} = Analyte concentration of the spiked sample
C_i = Initial analyte concentration of the un-spiked sample.
C_{added} = Analyte concentration added to the sample.

10.8. Other methods

- Calibration curve should have a correlation coefficient ≥0,995.
- The equipment performance (balance, pipettes, others) should be verified periodically for quality assurance of the results. A once-a-year calibration procedure is also advised (if possible, performed by an accredited calibration company). Regarding balances, a daily verification procedure is recommended (using certified calibration weights).
- The analysis of blind samples and duplicate samples within an analysis run is recommended, at a frequency established by each laboratory under their internal procedures.

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Appendix I – Moisture content correction factor

For the equation 1, the Fh term can be obtained from the soil moisture content determination. This factor can be calculated as expressed in equation 2:

$$Fh = \frac{M_{cds} - M_c}{M_{cms} - M_c} \quad (2)$$

Where:

Fh = humidity correction factor (dry-weight fraction)

Mcms = mass of container and moist soil, in g

Mcds = mass of container and oven-dry specimen, in g

Mc = mass of container, in g

Appendix II – Determination of phosphorous by colorimetry

The Sensitivity of ICP OES to P is about 0.5 mg/L. Therefore, if 1 g of sample and a 50 mL container are used, the final quantity of analyte should be about 25 mg/kg. That is acceptable for most soils, but if lower detection limits are required it may be necessary to use a colorimetric procedure employing the phosphomolybdate reaction.

Materials

- Ammonium molybdate;
- Potassium antimony tartrate;
- Ascorbic acid;
- Ethyl alcohol;
- 4% ammonium molybdate: hot dissolve 4 g of molybdate in 50 mL of demineralized water, let cool then adjust to 100 mL;
- Potassium antimony tartrate at 0.3%: dissolve 0.3 g of potassium antimony tartrate in 50 mL of demineralized water then adjust to 100 mL;
- Colour reagent 1: add 50 mL of 2N sulfuric acid, 15 mL of 4% ammonium molybdate, 5 mL of 0.3% potassium antimony tartrate; adjust to 100 mL with deionized water;
- Colour reagent 2: dissolve 2 g of ascorbic acid in 50 mL of demineralized water; add 25 mL of ethyl alcohol, 5 mL of concentrated HCl; adjust to 100 mL with deionized water;
- Reagent: pour solution 2 into solution 1 (yellow color).

Procedure

The sample's amount of P is quantified on the extract obtained at point 8.1 or 8.2 using a spectrophotometer (suitable for measuring absorbance at about 627 nm), or continuous flow analyzer.

For continuous flow analyzer, the following flows are suggested:

- extract: 0.16 mL/min
- water: 1.60 mL/min
- reagent: 0.32 mL/min

The calibration curve range is prepared from the 1 g/L P solution with 7 points ranging from 0 to 10 mg/L and must be adjusted according to the content of the samples. For reagents see section 5.3.

Appendix III – Standard preparation

For calibration curves and quality control standards, the following procedure can be followed as an example:

- **Standard phosphate solution, 100 mg P/L.** Pipette 50 mL of NIST or other equivalent traceable 1000 mg P/L phosphorus stock solution into a 500 mL volumetric flask and make up to volume with the extracting solution. Alternatively, dissolve 0.4390g of KH_2PO_4 (dried for two hours at 110 °C) in the extracting solution in a one liter volumetric flask and make up to the final volume.
- **Secondary standard phosphate solution, 4 mg P/L.** Pipette 10.0 mL of 100 mg P/L standard solution into a 250 mL volumetric flask and make up to the final volume with the extracting solution.
- **Working phosphate standard series.** Pipette into 100 mL volumetric flasks 0, 10, 20, 30, 40 and 50 mL of the 4 mg P/L standard solution. Make up to the final volume with the extracting solution. The standard series is then 0, 0.4, 0.8, 1.2, 1.6 and 2.0 mg P/L.

The range for working phosphate standards can be adjusted according to the equipment specification and the expected concentration of P in the analyzed soil samples.

Appendix IV - Acknowledgments

GLOSOLAN would like to thank Ms Laura Marcela Casas Cevallos from Colombia and Ms Marie-Pierre Tine from Senegal for leading the harmonization of this SOP and the members of the working group that served as leaders for their regions and contributed to the writing of this SOP (appendix V).

GLOSOLAN would also like to thank the experts who were part of the Review Panel and who ensured the finalization of the SOP (appendix V), all the laboratories that provided inputs for the harmonization of this method (appendix VI), all the experts from the GLOSOLAN Technical Committee and from the International Network on Soil Pollution (INSOP) who technically reviewed this SOP.

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Appendix VI - Contributing laboratories

GLOSOLAN thanks the following laboratories for completing the GLOSOLAN form on the method and providing information on their standard operating procedure for quasi total elements in soil by acid digestion, including heavy metals. This information was used as a baseline for the global harmonization.

From the African Region:

- Laboratoire de Pédologie de l'Université de Kinshasa (LABOPEDO UNIKIN), **Democratic Republic of Congo**
- Triomf Swaziland PTY LTD, **Eswatini**
- Department of Soil Science, Institute for Agricultural Research - Ahmadu Bello University Samaru Zariay, **Nigeria**
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From the Asian Region:

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- DA Regional Field Office 3-ILD-Regional Soils Laboratory, **Philippines**
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From the Eurasian region:

- Institute of Biology of Komi Science Centre of the Ural Branch of the Russian Academy of Sciences (IB Komi SC UB RAS), **Russian Federation**

[1]References to Kosovo shall be understood to be in the context of Security Council resolution 1244 (1999).

From the European Region:

- Environment Agency Austria (EAA), **Austria**
- Central Institute for Supervising and Testing in Agriculture, **Czechia**
- Estonian Environmental Research Center, **Estonia**
- National Food Chain Safety Office, Food Chain Safety Laboratory Directorate - Plant Protection and Soil Conservation National Reference Laboratory, Soil Conservation Department, **Hungary**
- Kosovo Institute of Agriculture, **Kosovo**
- Latvian State Forest Research Institute "Silava", **Latvia**
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From the Latin America Region:

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- Agencia de Regulación y Control Fito y Zoosanitario – AGROCALIDAD, **Ecuador**
- FUSADES, **El Salvador**
- Lab.Quimico Agricola y Residuos de pesticidas-FHIA, **Honduras**
- Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Tecomán, **Mexico**
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- Laboratorio de Análisis de Suelos y Agua - Instituto de Geología (UNAM), **Mexico**

From the Near East and North African Region:

- Soils, Water and Environment Research Institute (SWERI), **Egypt**
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- National Institute of Agricultural Research (INRA), **Morocco**
- DRS/DGACTA, **Tunisia**

From the North America region:

- Kellogg Soil Survey Laboratory (USDA-NRCS-NSSC), **United States of America**

From the Pacific Region:

- Analaytical Laboratories and Technical Services, **Australia**
- CSBP Soil and Plant Lab, **Australia**
- Dual Chelate Fertilizer Pty Ltd, **Australia**
- Swep Analytical Laboratories, **Australia**
- Unitech Analytical Services Laboratory - University of Technology, **Papua New Guinea**

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The Global Soil Partnership (GSP) is a globally recognized mechanism established in 2012. Our mission is to position soils in the Global Agenda through collective action. Our key objectives are to promote Sustainable Soil Management (SSM) and improve soil governance to guarantee healthy and productive soils, and support the provision of essential ecosystem services towards food security and improved nutrition, climate change adaptation and mitigation, and sustainable development.

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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