



Food and Agriculture  
Organization of the  
United Nations

**Standard operating procedure  
for soil available micronutrients  
(Cu, Fe, Mn, Zn)  
and heavy metals (Ni, Pb, Cd)**

**DTPA extraction method**

**GLOSOLAN**

GLOBAL SOIL LABORATORY NETWORK



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Available micronutrients (Cu, Fe, Mn, Zn) and heavy metals (Ni, Pb, Cd), DTPA extraction method	Version number : 1	Page 1 of 11
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## **SOIL AVAILABLE MICRONUTRIENTS AND HEAVY METALS DTPA extraction method**

### VERSION HISTORY

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01	12 April 2022	All comments by RESOLANs and reviewers to the draft SOP were addressed	Finalization of the SOP
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04			
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## 1. Brief introduction to micronutrients and heavy metals

Copper, iron, manganese and zinc are soil micronutrients which are essential for plant growth and living organisms.

Copper is involved in many enzyme pathways including those involved in N fixation and DNA/RNA synthesis which affects reproduction. Copper is less mobile than other micronutrients and has a higher risk of accumulating to harmful concentrations due to Cu-based fungicide use and other human activities.

Iron is involved in all enzyme systems that contain the heme group of biochemical including chlorophyll production and is one of the main elements that gives soil its color. Well-aerated and alkaline soils generally have low plant-available iron content, whereas soils rich in organic carbon normally have higher iron content. Waterlogging of soils can result in iron availability so high as to be toxic to plants. In higher concentrations, iron can badly affect the availability of other plant nutrients, and contrarily its availability is decreased by high zinc content.

Manganese is involved in photosynthesis and facilitates enzyme activity. It can easily oxidize or reduce and its availability is significantly affected by soil pH and Eh therefore acid waterlogged soils often show toxic levels of plant-available manganese.

Zinc is involved in many enzyme systems including RNA polymerase, several dehydrogenases and influences the production of auxins. Its concentration is influenced by soil texture, thus sandy soils have lower zinc content, while clay soils have a higher content. Zinc availability is affected by soil pH, similarly to manganese, and by soil phosphorus content. Zinc and copper have an antagonistic relationship.

Nickel, lead and cadmium are potentially toxic elements, which can have natural origins, but high and harmful amounts are often a result of human activity. At higher concentrations, these elements can have negative consequences to human health.

## 2. Scope and field of application

The method provides a potential indicator of the plant available copper, iron, manganese and zinc in soil. It can be used to extract heavy metals such as nickel, lead and cadmium assisting with conclusions as to their potential bioavailability. This method must be used in combination with suitable determination methods, for example, flame atomic absorption spectrometry, electrothermal atomic absorption spectrometry or inductively coupled plasma – optical emission spectrometry.

This method is applicable to all types of soils but has not been correlated with plant uptake in all types of soils and with all crop or plant species and varieties. To improve the interpretation of plant

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availability in novel soil and plant species it is recommended to use plant tissue analysis as a further diagnostic tool.

### 3. Principle

The DTPA soil test was developed by Lindsay and Norvell (1978) to identify near-neutral and calcareous soils with insufficient available Zn, Fe, Mn, or Cu for maximum yields of crops. The soil sample is extracted in a DTPA, TEA and CaCl<sub>2</sub> solution at 25 °C temperature. The extraction solution is buffered to 7.3 ± 0.2 to ensure that samples pH does not affect the extraction. DTPA is a chelating agent that forms complexes with the determinable elements. TEA functions as part of the buffer solution and masking reagent that forms complexes with aluminum and other interfering elements. Also, TEA burns clearly during atomization of extractant solution while being measured on the AAS. The presence of 0.01 M CaCl<sub>2</sub> enables the extract to attain equilibrium with CaCl<sub>2</sub>, which minimizes the dissolution of CaCO<sub>3</sub> from calcareous soils. The extraction conditions must be strictly observed to give comparable results. Temperature, shaking time and shaking speed can influence the reaction kinetics and formation of the complexes.

### 4. Apparatus

- 4.1. Sealable sample vessel with at least 50 mL or 100 mL nominal volume.
- 4.2. Analytical balance with 0.01 g resolution.
- 4.3. End-over-end shaker with 30 rpm rotation speed and 50 cm rotation amplitude or reciprocating shaker with 120 rpm rotation speed and 8 cm rotation amplitude.
- 4.4. Filter vessels with at least 30 mL nominal volume.
- 4.5. Fine-porosity filter paper (Whatman No. 42 or equivalent).
- 4.6. Flame atomic absorption spectrometer (FAAS), electrothermal atomic absorption spectrometer (ETAAS) or inductively coupled plasma – optical emission spectrometer (ICP-OES).

#### Optionally:

- 4.7. Centrifuge with 3000 rpm rotation speed.
- 4.8. Sealable centrifuge tubes with 50- or 100-mL nominal volume.

### 5. Materials

All reagents should at least be Analytical Grade.

- 5.1. Deionized water/distilled water, with specific conductivity not higher than 2 μS m<sup>-1</sup> at 25 °C and a pH greater than 5.6 (or grade 2 water or type II water according to ISO 3696 and ASTM D1193-06, respectively, if this is the quality of water produced in the laboratory).
- 5.2. Diethylenetriaminepentaacetic acid (DTPA, C<sub>14</sub>H<sub>23</sub>N<sub>3</sub>O<sub>10</sub>).
- 5.3. Triethanolamine (TEA, C<sub>6</sub>H<sub>15</sub>NO<sub>3</sub>).

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- 5.4. Calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ).
- 5.5. Hydrochloric acid (HCl) or hydrochloric acid solution of any concentration.
- 5.6. DTPA extraction solution,  $c(\text{DTPA}) = 0.005 \text{ mol L}^{-1}$ ,  $c(\text{TEA}) = 0.1 \text{ mol L}^{-1}$ ,  $c(\text{CaCl}_2) = 0.01 \text{ mol L}^{-1}$ .
- 5.7. Dissolve 1.967 g DTPA (5.2), 14.919 g TEA (5.3.) and 1.470 g  $\text{CaCl}_2$  (5.4.) in 900 mL deionized water (5.1.). Adjust the pH to  $7.3 \pm 0.2$  hydrochloric acid or hydrochloric acid solution (5.5.) (for reference, approximately 8.3 mL of 1:1 HCl solution is required). Bring the final volume to 1000 mL with deionized water (5.1.). Can be stored up to 3 months, checking that pH is within the acceptable limit before each use.
- 5.8. Single element or multiple element standards.

## 6. Health and safety

This SOP involves the use of hazardous chemicals. Refer to the laboratory safety guidelines or the Safety Data Sheet (SDS) before proceeding

### 6.1. Personnel safety

Wear proper personal protective equipment. Use a laboratory coat, closed shoes, a gas or dust mask, appropriate gloves, and safety glasses when performing chemical analysis to mitigate the harmful effects of chemical exposure. Wash hands and other exposed areas with mild soap and water after using all chemical reagents.

### 6.2. Chemical hazard

- 6.2.1. DTPA is a white solid. DTPA is irritant and a health hazard. DTPA causes serious eye irritation and damage to organs through prolonged or repeated exposure. DTPA is harmful when inhaled.
- 6.2.2. TEA is an oily liquid with a mild ammonia odor. TEA is corrosive, irritant and a health hazard. TEA causes skin irritation, serious eye damage or irritation, and damage to organs through prolonged or repeated exposure. TEA is harmful if swallowed.
- 6.2.3. Hydrochloric acid is a colorless watery liquid with a sharp, irritating odor. Hydrochloric acid is corrosive and acutely toxic. Hydrochloric acid causes severe skin burns and eye damage. Hydrochloric acid is toxic if inhaled.

## 7. Sample preparation

Use an air dried (or dried in a ventilated drying oven at a temperature below  $40^\circ\text{C}$ ) soil sample, which is ground to pass a 2 mm aperture sieve. Follow GLOSOLAN-SOP-01 "Standard operating procedure for handling and preparation of soil samples for chemical and physical analyses" wherever possible.

Ensure that the milling equipment, sieves and all the materials used do not introduce contamination to the samples.

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## 8. Procedure

### 8.1. Extraction

- 8.1.1. Weigh 10.0 g of sample (4.2.) in the sample vessels (4.1.).
- 8.1.2. Add 20.0 mL DTPA extraction solution (5.6.)
- 8.1.3. Shake for exactly 2 hours at 25 °C (4.3.).
- 8.1.4. Filter the extract to filtrate vessel (4.4.) by passing it through fine-porosity filter paper (4.5.), discarding the first portion of the filtrate. Optionally decant the filtrate or the unfiltered extract to centrifugation tubes (4.8.) and centrifugate it at 3000 rpm (4.7.) until clear (nominally 10 minutes).

#### Points to be noted

- In each batch include at least 1 blank.
- For soil with high organic content or absorbing capacity, there is possibility that the volume of the extract is not enough for analysis. In such cases weigh 20.0 g of sample and add 40.0 mL DTPA extraction solution instead, using labware of suitable volume.
- The extract must be analyzed within 48 hours.
- If using both filtering and centrifugation, it is advantageous if the filtrate vessel (4.4.) is also the centrifugation tube (4.8.).

### 8.2. Determination

- 8.2.1. Calibrate the measuring instrument (4.6.) using single element standards or multiple element standards (5.8.) following the manufacturer's recommendations for operation and calibration of the instrument.
- 8.2.2. Analyze the extracts following the manufacturer's recommendations for operation of the instrument. The extract should be diluted (with extraction solution) if the sample concentration exceeds the concentration of the highest standard.

## 9. Calculation

Report the results in milligrams of element per kilogram of soil ( $\text{mg kg}^{-1}$ ).

The test report should include:

- The reference to this SOP;
- The result of the determination, with 2 decimal places.

## 10. Quality assurance / quality control

The described quality control measures should be considered minimal requirements. If possible and necessary, use stricter quality control measures.

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### 10.1. Precision test

Perform replicate analysis every 10 samples in each batch test. For batches smaller than 10 samples include 1 sample. Calculate the percent relative standard deviation (% RSD) to determine precision.

$$\% RSD = \frac{s}{\bar{x}} \times 100$$

Where: s = standard deviation of the replicate result

$\bar{x}$  = mean

The acceptance requirements for precision testing must be defined by the equipment used, environmental conditions, and other testing factors and by the specifications or requirements for the information use and agronomic criteria.

If the precision test fails, the cause must be identified and corrective or preventive actions must be developed. Record any actions taken.

### 10.2. Interlaboratory Proficiency Test

Participate in an Interlaboratory Proficiency Test at least once a year. The PT z-score should be less than 2. If it is not, the cause must be identified and corrective or preventive actions must be developed. Record any actions taken.

### 10.3. Control chart

Plot replicate sample-data in a control chart to observe whether values are in the confidence interval and monitor results. If results are out of the specified limits (or tend to be so), an evaluation must be made. The cause must be identified, and corrective and preventive actions must be developed. Record any actions taken.

## 11. Reference documents

**ISO 14870:2001.** *Soil quality — Extraction of trace elements by buffered DTPA solution. 2001.*

**Lindsay, W.L. and Norvell, W.A. (1978).** *Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Sciences Society of America Journal 42: 421-428. 1978.*

**Page, A.L. et al. (Eds.)** *Methods of Soil Analysis, Number 9, Part 2, Chemical and Microbiological Properties, Second Edition, American Society of Agronomy, Madison, Wisconsin. 1982.*

**Rayment, G.E., and D.L. Lyons (2011)** *Soil Chemical Methods – Australasia.* CSIRO Publishing. Collingwood VIC.

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## 12. Annex I. – Acknowledgments

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## 13. Annex II. – List of authors

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- Simon Leake, Sydney Environmental and Soil Laboratory Pty Ltd, **Australia**
- My Chi Mai, Sydney Environmental and Soil Laboratory Pty Ltd, **Australia**
- Karim Shahbazi, Soil and Water Research Institute Laboratory, **Iran**

## 14. Annex III. – Contributing laboratories

GLOSOLAN thanks the following laboratories for completing the GLOSOLAN form on the method and providing information on their standard operating procedure for the soil pH determination method. This information was used as a baseline for the global harmonization.

### From the African region:

- Soil and Plant Analytical Laboratory - Department of Agricultural Research, **Botswana**
- Laboratoire de Pédologie de l'Université de Kinshasa - Labopedo Unikin, **Democratic Republic of Congo**
- Analytical Services Laboratory - Soil Research Institute, **Ghana**
- Laboratoire Sol-Eau-Plante LPCM/LSEP, **Mali**

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From the Asian region:

- Central Laboratory, Soil Resource Development Institute, **Bangladesh**
- Agri-Biochem Research Lab, **India**
- Charles Renard Analytical Laboratory - International Crops Research Institute For The Semi-Arid Tropics (ICRISAT), **India**
- ICAR-Indian Institute of Soil Science, **India**
- Laboratorium Pengujian, Balittanah - Soil Test Laboratory, Indonesian Soil Research Institute (ISRI), **Indonesia**
- Soil Science, Water Utilization and Agricultural Engineering Division in the Department of Agricultural Research (DAR), **Myanmar**
- Soil Science Division, Nepal Agricultural Research Council, **Nepal**
- Bureau of Soils and Water Management - Laboratory Services Division (BSWM-LSD), **Philippines**
- Department of Agriculture - Regional Field Office 3-ILD – Regional Soils Laboratory, **Philippines**
- Department of Agriculture - Regional Soils Laboratory 4a, **Philippines**
- Department of Agriculture - Regional Field Office Cordillera Administrative Region - Regional Soils Laboratory DA-RFO CAR, **Philippines**
- Soil Chemistry Research Group, Office of Science for Land Development (OSLD) - Land Development Department (LDD), **Thailand**

From the European region:

- Laboratório de Solos e Plantas da UTAD, **Portugal**
- Laboratorio Químico Agrícola Rebelo da Silva - Instituto Nacional de Investigação Agrária e Veterinária (INIAV), **Portugal**
- National Research and Development Institute for Soil Science Agrochemistry and Environment – Department for physical and chemical analysis (INCDPAPM-ICPA), **Romania**
- Department of Laboratory Methods of Soil Science and Conservation Research Institute SSCRI, **Slovakia**
- Testing Laboratory of Soil and Fertilizer Analysis - Central Control and Testing Institute in Agriculture, **Slovakia**
- Soil Quality Assessment - Research Group Soil Quality Assessment, Universidade de Santiago de Compostela, **Spain**
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- Soil, Water, Plant, Fertilizer Laboratory - International Agricultural Research And Training Center (IARTC-UTAEM), **Turkey**

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- Laboratory of Chemical and Biological Factors, **Ukraine**

From the Latin American region:

- Laboratorio de Suelo y Agua, Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Anguil, **Argentina**
- Laboratorio de Analise de Agua, Solo e Planta - Embrapa, **Brazil**
- Laboratorio de Ecología Microbiana y Biogeoquímica de Suelos, Institute of Agri-Food, Animal and Environmental Sciences (ICA3) - Universidad de O'Higgins, **Chile**
- Laboratorio de Suelos y Plantas INIA Centro Sur, INIA Quilamapu - Instituto de Investigaciones Agropecuarias, **Chile**
- Instituto Geográfico Agustín Codazzi (IGAC), **Colombia**
- Laboratorio de FUSADES, **El Salvador**
- Laboratorio Químico Agrícola & Residuos de Pesticidas de la Fundación Hondureña de Investigación Agrícola (FHIA), **Honduras**
- Biosollo S.R.L, **Paraguay**
- Laboratorio Agroindustrial, Suelos, Plantas y Aguas, Colegio de Postgraduados, **Mexico**
- Laboratorio de análisis de suelos, agua y plantas de Instituto de Ecología - A. C., **Mexico**
- Laboratorio de Diagnóstico Ambiental del Instituto Tecnológico del Valle de Oaxaca, **Mexico**
- Laboratorio de Edafología Ambiental - Instituto de Geología (UNAM), **Mexico**
- Laboratorio de Fertilidad de Suelos y Química Ambiental - Colegio de Postgraduados, Campus Montecillo (LABFER - CPM), **Mexico**
- Laboratorio de Química y Contaminación de Suelos, Aguas y Plantas – Lab DICA, **Mexico**
- Laboratorio de Suelo, Agua y Planta - INIFAP Campo Experimental Tecomán, **Mexico**
- Department of Food Production Soils Laboratory - The University of the West Indies, St. Augustine, **Trinidad and Tobago**
- Laboratorio de Suelos, Plantas y Agua - - Instituto Nacional de Investigación Agropecuaria (INIA), **Uruguay**

From the Near East and North African region:

- KIMIA AB Environmental and Agricultural Consulting Laboratory, **Iran**
- Parham Gostar Laboratories, **Iran**
- Soil and Water Lab., Desert Studies Center - University of Anbar, **Iraq**

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- Soil Fertility and Fertilizers - Ministry of Science and Technology, Directorate of Agricultural Research, **Iraq**
- Soil Physical Properties and Water Management Laboratory - Soil and Water Resources Centre, Directorate of Agricultural Researches, Ministry of Science and Technology, **Iraq**
- Soil Chemistry and Physics Laboratories - Kuwait institute for scientific research, **Kuwait**
- Soil Characterization Lab - AUB, **Lebanon**
- INRA (National Institute of Agricultural Research), **Morocco**
- Labomag A bureau veritas Group Company, **Morocco**
- Damascus lab (ANRR-lab1) - General Commission of Sciences Agriculture Research (GCSAR), **Syria**
- Homs lab (ANRR-lab6) - General Commission of Sciences Agriculture Research (GCSAR), **Syria**
- Lab of Alhanadi research station - General Commission of Sciences Agriculture Research (GCSAR), **Syria**
- DRS/DGACTA, **Tunisia**

From the Pacific region:

- CSBP Soil and Plant Laboratory, **Australia**
- Department of Environment and Science (DES), Science Division, Chemistry Centre, **Australia**
- Dual Chelate Fertilizer Pty Ltd, **Australia**
- Nutrient Ag Solutions, **Australia**
- SWEP Pty Ltd Analytical Laboratories, **Australia**
- Laboratoire des Moyens Analytiques Nouméa – IRD, **New Caledonia**
- QLabs Ltd, **New Zealand**
- Unitech Analytical Services Laboratory – University of Technology, **Papua New Guinea**
- School of Agriculture and Food Technology - The University of the South Pacific, **Samoa**

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**GLOSOLAN  
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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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