TEST METHODS MANUAL

Laboratory Services Division Bureau of Soils and Water Management

TM-LSD-04-09

Department of Agriculture		
SECTION : SOIL CHEMISTRY	Issue No.: 3	Effective date: April 15, 2024
SUBJECT : AVAILABLE Cu, Zn, Fe and Mn (DTPA-TEA EXTRACTION - ATOMIC ABSORPTION SPECTROSCOPY METHOD)	Revision No.: 1	Page 1 of 7

SCOPE

This method is for the determination of plant available Cu, Zn, Fe, and Mn in soils using an Atomic Absorption Spectrophotometer (AAS).

The Diethylene triamine pentaacetic acid-triethanolamine (DTPA-TEA) soil test was developed to identify near neutral and calcareous soils with insufficient levels of Cu, Zn, Fe, and Mn.

PRINCIPLE

Micronutrients are essential elements that are used by plants in small quantities. Yield and quality of agricultural products increased with micronutrients application, therefore human and animal health is protected with feed of enrichment plant materials. Micronutrients include Cu, Zn, Fe, Mn and the available forms of these are their cations, Cu²⁺, Zn²⁺, Fe²⁺ and Mn²⁺, respectively.

Chelating agents offer great promise for assessing readily micronutrient cations in soils. DTPA is one of the most commonly used because it offers the most favorable combination of stability constants for simultaneous complexation, and measurements of Cu, Zn, Fe, and Mn. The extractant was designed specifically to avoid excessive dissolution of $CaCO_3$ with the release of occluded micronutrients, which are normally not available for absorption by roots. This was achieved by buffering the extracting solution in a slightly alkaline pH range and by including soluble Ca^{2+} . Triethanolamine was selected as the buffer because of its pK_a (7.8) and because it burns cleanly with little interference during AAS analysis.

Experimental conditions such as shaking time, shaking speed, concentration of DTPA, pH, and temperature, influence the amount of Cu, Zn, Fe, and Mn extracted by DTPA.

An extraction time of 2 hours with a speed of 120 cycles per minute (cpm) was chosen to ensure that the initial rapid dissolution of the micronutrient would be complete and slight variations in time of preparation and filtration would not be critical.

A DTPA concentration of 0.005 M was selected because this concentration provides ample chelating capacity to remove measurable amounts of all four micronutrient metals and provided sufficient excess to prevent competitive secondary interactions among the metals extracted.

The pH 7.3 is within the natural range of near-neutral and alkaline soils, and affords rapid equilibrium with $CaCO_3$ at a CO_2 partial pressure approximately 10 times that of the atmosphere. At higher pH values, too little Fe is dissolved to measure conveniently while at lower pH values, too much Fe and Mn are potentially extractable, and $CaCO_3$ is unstable.

Increasing temperature increases the extractable micronutrients, thus 23-25°C is adopted. Standardization of sample preparation and extraction procedures is absolutely essential.

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EXTRACTION - ATOMIC ABSORPTION SPECTROSCOPY		
METHOD)		

TEST PRECAUTIONS

Micronutrient contamination of samples can occur quite easily if care is not taken in collection and preparation of samples. Soil probes, sample containers and soil grinding equipment should all be checked for potential contamination before being used. Additional care in laboratory techniques over those used for the macronutrient tests must be taken within the laboratory in preparing all reagents, cleaning glassware and selecting reagents to avoid contamination.

The amount of Cu, Zn, Fe, and Mn extracted by DTPA is highly dependent on experimental conditions such as shaking time, shaking speed, concentration and pH of the extracting solution and temperature. Thus, standardization of the procedure is very important to obtain uniform and reproducible results in the DTPA soil test.

Do not reuse pipettor tips to prevent cross contamination. Ensure that the burner is clean prior to analysis. Check if there are foreign materials blocking sipper. If the sipper is clogged, clean the sipper using the wire provided by the manufacturer.

Dispensing of extracting solution from bottle top dispenser to soil samples must be done carefully to prevent accumulation of soil particles in the dispensing nozzle of bottle top dispenser that may drop off in the next receiving vessel and results in contamination of soil sample. If residues or soil particles were observed, clean the dispensing nozzle of bottle top dispenser by wiping it with a piece of tissue paper.

If the sample is highly diluted, repeat the analysis for the specific sample and reduce the weight of sample.

EQUIPMENT

- a) Analytical Balance
- b) pH Meter
- c) Reciprocal Shaker
- d) Centrifuge
- e) AAS
- Water Deionizer f)
- g) Vortex Mixer

LABORATORY WARE

- a) Polyethylene centrifuge tubes, 50 mL
- b) Centrifuge racks
- c) Test tubes (10mL, 20mL, 30 mL)
- d) Test Tube racks
- e) Filter funnels
- f) Bottle top dispenser (1L)
- g) Pipettor (10mL)
- h) Polyethylene container, 5L
- i) Vials, 50 mL
- Graduated Cylinder (100 mL) i)

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k) Beakers (100 mL, 250 mL, 500 mL)

- I) Volumetric flasks (100 mL, 200 mL)
- m) Whatman No. 42 filter paper

CHEMICALS AND REAGENTS

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Form.

Note: Small quantities of white precipitates will likely remain in the bottom of the reagent bottle when creating the DTPA extract. This material is insolu Google Form link: A reagent and will not pose an analytical issue provided the precipitates/forms.gle/RbCgCdA54prTS60N7^{ation.} The extracting solution can be filtered if precipitates become a problem of the dispension.

This solution is stable for several months provided the laboratory takes steps to prevent evaporation and limits exposure of the solution to sunlight. The pH should be checked periodically before use because a pH of 7.3 is critical for the extraction.

b) Hydrochloric acid, 6.0 M. In a 200mL volumetric flask transfer 50mL of deionized water then add 100mL concentrated HCl. Mix the flask containing the Thank You! flask up to mark with deionized water.

Note: Concentrated HCl is a strong acid which produce fumes that is harmful to our health, work under a fume hood and wear personal protective equipment when preparing the solution. Diluting concentrated HCl with deionized water undergoes exothermic reaction, cool the solution before filling up to mark. When diluting strong acids, always add acid into water to prevent violent reaction.

- c) NIST Traceable Standard of Cu, Zn, Fe, Mn Standards, 1000 mg/L
- d) Working Standards: Standards should be diluted with the DTPA extracting solution. Concentration range for standards should cover Cu, 0 to 5 mg/L; Zn, 0 to 1 mg/L; Fe, 0 to 5 mg/L; and Mn, 0 to 5 mg/L.

HEALTH AND SAFETY

Eyes are especially vulnerable to splashes from reagents and therefore, safety spectacles should always be worn when carrying out any steps involving risk of such splashing. Skin absorption can be protected by wearing lightweight disposable gloves while weighing and handling of chemicals. Heavy duty gloves must be worn when handling corrosive substances. Cotton made laboratory aprons should be used to protect from chemical splashes and face masks are necessary while working with hazardous chemicals.

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