

## 5. ANALYTICAL PROTOCOL FOR EXCHANGEABLE CALCIUM AND MAGNESIUM IN SOIL (VERSENATE METHOD)

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### a. Estimation of Ca and Mg in soil

**Principle:** Exchangeable cations are usually determined in a neutral normal ammonium acetate extract of soil. Extraction is carried out by shaking the soil: extractant mixture followed by filtration or centrifugation. Calcium and magnesium are determined either by EDTA titration method or by atomic absorption spectrophotometer after the removal of ammonium acetate and organic matter. It may be noted that in soils appreciable amount of soluble calcium and magnesium may be present. Hence, these water soluble cations are estimated in the 1:2 soil water extract and deducted from ammonium acetate extractable calcium and magnesium (since ammonium acetate also extracts water soluble cations) to obtain exchangeable Calcium and magnesium.

The EDTA titration method developed by Chang and Bray (1951) is preferred on account of its accuracy, simplicity and speed. The method is based on the principle that calcium, magnesium and a number of other cations form stable complexes with versenate (ethylenediamine tetraacetic acid disodium salt) at different pH. The interference of Cu, Zn, Fe, Mn is prevented by the use of 2% NaCN solution or carbamate. Usually in irrigation waters and water extracts of soil, the quantities of interfering ions are negligible and can be neglected. A known volume of standard calcium solution is titrated with standard versenate 0.01N solution using murexide (ammonium purpurate) indicator in the presence of NaOH solution. The end point is a change of colour from orange red to purple at pH 12 when the whole of calcium forms a complex with EDTA.

### **a. Calcium by Versenate (EDTA) method**

#### *Apparatus*

- Shaker
- Porcelain dish
- Beakers

## Training Manual on "Soil Testing and Fertilizer Recommendation"

- Volumetric/conical flask.

### **Reagents**

- Standard 0.01N calcium solution: Take accurately 0.5 g of pure calcium carbonate and dissolve it in 10 ml of 3N HCl. Boil to expel CO<sub>2</sub> and then make the volume to 1 L with distilled water.
- EDTA solution (0.01N): Take 2.0 g of versenate, dissolve in distilled water and make the volume to 1 litre. Titrate it with 0.01N calcium solution and make necessary dilution so that its normality is exactly equal to 0.01N.
- Muroxide indicator powder: Take 0.2 g of muroxide (also known as ammonium purpurate) and mix it with 40 g of powdered potassium sulphate. This indicator should not be stored in the form of solution, otherwise it gets oxidized.
- Sodium diethyl dithiocarbamate crystals: It is used to remove the interference of other metal ions.
- Sodium hydroxide 4N: Prepare 16% soda solution by dissolving 160 g of pure sodium hydroxide in water and make the volume to 1 L. This will give pH 12.

### **Procedure**

1. Take 5 g air dried soil sample in 150 mL conical flask and add 25 mL of neutral normal ammonium acetate. Shake on mechanical shaker for 5 minutes and filter through Whatman filter paper No.1.
2. Take a suitable aliquot (5 or 10 mL) and add 2-3 crystals of carbamate and 5 mL of 16% NaOH solution.
3. Add 40-50 mg of the indicator powder. Titrate it with 0.01N EDTA solution till the colour gradually changes from orange red to reddish violet (purple). It is advised to add a drop of EDTA solution at an interval of 5 to 10 seconds, as the change of colour is not instantaneous.
4. The end point must be compared with a blank reading. If the solution is over titrated, it should be back titrated with standard calcium solution and exact volume used is thus found.
5. Note the volume of EDTA used for titration.

### **Calculation**

If  $N_1$  is normality of Ca<sup>++</sup> and  $V_1$  is volume of aliquot taken and  $N_2V_2$  are the normality



lower valency state (Mn<sup>2+</sup>). Or mix thoroughly 0.5 g of the indicator with 50 g of ammonium chloride.

- Sodium cyanide solution (2%) or sodium diethyl dithiocarbamate crystals. This is used to remove the interference of copper, cobalt and nickel.

**Procedure**

1. Take 5 g air dried soil in 150 mL flask, add 25 mL of neutral normal ammonium acetate solution and shake on a mechanical shaker for 5 minutes and filter through Whatman No.1 filter paper.
2. Pipette out 5 mL of aliquot containing not more than 0.1 meq of Ca plus Mg. If the solution has a higher concentration, it should be diluted.
3. Add 2 to 5 crystals of carbamate and 5 mL of ammonium chloride-ammonium hydroxide buffer solution. Add 3-4 drops of Eriochrome black T indicator.
4. Titrate this solution with 0.01N versenate till the colour changes to bright blue or green and no tinge of wine red colour remains.

**Calculation**

If N<sub>1</sub> and V<sub>1</sub> are normality (concentration of Ca<sup>2+</sup> + Mg<sup>2+</sup>) and volume of aliquot taken and N<sub>2</sub>V<sub>2</sub> are the normality and volume of EDTA used respectively, then,

$$N_1V_1 = N_2V_2$$

$$\text{Or } N_1 = \frac{N_2V_2 \times \text{Normality of EDTA} \times \text{Vol of EDTA}}{V_1 \text{ mL of aliquot taken}}$$

Here N<sub>1</sub> (Normality) = equivalents of Ca<sup>2+</sup> plus Mg<sup>2+</sup> present in one litre of aliquot.

$$\text{Hence, Ca}^{+2} \text{ plus Mg}^{+2} \text{ me/litre} = \frac{\text{Normality of EDTA} \times \text{Vol of EDTA} \times 1000}{\text{mL of aliquot taken}}$$

Milliequivalent (me) of Mg<sup>++</sup> = me (Ca<sup>++</sup> + Mg<sup>++</sup>) – me of Ca<sup>++</sup>, When expressed on soil weight basis.

$$\text{Ca}^{++} + \text{Mg}^{++} \text{ me/100 g soil} = \frac{\text{Normality of EDTA} \times \text{Vol of EDTA} \times \text{Extract volume} \times 100}{\text{mL of aliquot taken} \times \text{Wt of soil}}$$

## **b. Estimation of Ca and Mg in Plant**

### ***Apparatus***

- Block-digester
- Porcelain dish
- Beakers
- Volumetric/conical flask.

### ***Reagents for digestion of plant sample***

1. Perchloric Acid ( $\text{HClO}_4$ ), 60%
2. Nitric acid ( $\text{HNO}_3$ )
3. Diacid mixture:  $\text{HNO}_3$  :  $\text{HClO}_4$  (9:4 ratio) mix 900ml of  $\text{HNO}_3$  with 400ml of  $\text{HClO}_4$

### ***Procedure for plant sample digestion***

1. Transfer 0.5 to 1.0 gm of plant sample into a 100ml conical flask or dry digestion tubes and wet the sample with 10ml of conc.  $\text{HNO}_3$ . Let it stand for atleast 2 hours or overnight. Pre digestion is required to avoid bumping or violent reaction on addition of diacid mixture to plant sample.
2. Then gently heat on a hot plate until the volume of the contents is reduced to about 4ml or even less and the material turns white sand.
3. Remove the flask from hot plate and allow it to cool. And add distilled water and make the volume upto 100 ml.

## **1. Estimation of Ca**

***Reagents:*** same as in soil analysis

### ***Procedure***

1. Pipette out 5 ml of the diacid or triacid extract into 50 ml conical flask.
2. Dilute to 20 - 30 mL with DI water, add 5 mL 4 N sodium hydroxide solution, and about 50 mg ammonium purpurate indicator.
3. Titrate with 0.01 N EDTA. The color change is from red to lavender or purple. Near the end point, EDTA should be added one drop every 10 seconds since the color change is not instantaneous.
4. Always run a blank containing all reagents but no sample, and treat it in exactly the same way

as the samples; and subtract the blank titration reading from the readings for all samples.

## 2. Estimation of Calcium plus Magnesium

**Reagents:** Same as in soil analysis

### **Procedure**

1. Pipette 10 mL aliquot into a 250-mL flask, dilute to 20 to 30 mL with DI water. Then add 3 - 5 mL buffer solution. And a few drops eriochrome black indicator.
2. Titrate with 0.01 N EDTA until the color changes from red to blue.

Calculation:

$$\text{Ca} = \frac{\text{Normality of EDTA} \times \text{Vol of EDTA} \times \text{Extract volume} \times 1000}{\text{mL of aliquot taken} \times \text{Wt of plant sample}}$$

$$\text{Ca} + \text{Mg} = \frac{\text{Normality of EDTA} \times \text{Vol of EDTA} \times \text{Extract volume} \times 1000}{\text{mL of aliquot taken} \times \text{Wt of plant sample}}$$

$$\text{Mg} = (\text{Ca} + \text{Mg}) - \text{Ca}$$

where Ca + Mg is expressed in meq/L basis