CHAPTER-III

Deeper investigation of Tabernaemontana coronaria
3.2 Soil Testing

The collected soil sample was investigated for the following parameters using standard procedures available in the literature.¹⁻⁵

➢ Determination of pH
➢ Determination of available carbon
➢ Determination of available phosphorous
➢ Determination of potassium content and
➢ Determination of micronutrients

3.2.1 Determination of pH of the Soil

pH of the soil is a measure of chemical and biochemical properties of the soil under investigation. It includes ion exchange, retention of ions by colloids, biological activity and nutrient availability in soil. Thus the soil pH assists in understanding the fertility status of the soil and also it is a parameter used in the classification of the soil into acidic and alkaline.

The pH of the soil was measured by Potentiometric method.

• Potentiometric method

Soil sample (10 g, 2 mm sieved) was mixed with distilled water (20 mL) and the mixture was stirred for half an hour. The glass electrode, serves as indicator electrode and calomel electrode, as reference electrode were immersed in the suspension of the soil and e.m.f of the cell, was recorded. The pH of the solution was calculated by using the following equation,

\[
pH = \frac{\text{E.m.f of the cell (E)}}{2.303 \cdot \frac{R \cdot T}{nF}}
\]

Where,
- \( R = \) Gas constant
- \( T = \) Temperature (Absolute)
n= Number of electrons
F= Faraday constant
E= Electrical potential of the cell

At 25°C, the equation (1) becomes

\[ \text{pH} = \frac{\text{E.m.f of the cell (E)}}{0.0591} \]

The e.m.f of the cell was found to be 0.36642, substituting this value in equation (2), we get,

\[ \text{pH} = \frac{0.36642}{0.0591} \]

\[ \text{pH} = 6.2 \]

Therefore, the pH of the soil under investigation was found to be 6.2. This value indicates that the soil sample was slightly acidic in nature.

3.2.2 Determination of organic carbon in soil

Nitrogen is an essential constituent of the plant, which they get from soil. Thus percentage of nitrogen in the soil, plays a significant role as far as fertility is concerned. Generally, carbon and nitrogen exist in a definite ratio of 10:1 in almost all soils. Hence, the determination of organic carbon in the soil, leads to the percentage of nitrogen in it. Generally, colorimetric method was adopted for the determination of organic carbon.

• Principle

Organic matter of the soil was oxidized by potassium dichromate solution in presence of dilute sulphuric acid. Potassium dichromate, being an oxidizing agent, oxidizes the carbon of the soil into carbon dioxide. The corresponding amount of potassium dichromate was reduced to green coloured chromium sulphate. The intensity of this colour so produced was dependent on the amount of organic carbon present in
the soil. Thus, by measuring the amount of chromium sulphate formed, by colorimetric method, the amount of carbon and hence nitrogen can be determined.

\[
K_2Cr_2O_7 + 4H_2SO_4 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + 4H_2O + 3[O]
\]

\[
3C + 6[O] \rightarrow 3CO_2
\]

- **Colorimetric method**

The procedure for the determination of carbon content by colorimetric method involves the following steps:

- **Preparation of reagents**
  
  - Potassium dichromate solution (1N) was prepared by dissolving potassium dichromate (29.04 g) in water (1000 mL)
  
  - Standard sucrose solution was prepared by dissolving sucrose (0.5 g) in distilled water (1000 mL)

- **Standard curve**

  Standard curve was obtained by oxidizing the carbon content in sucrose. Accurate volumes of standard sucrose solution, i.e. 0, 1, 2, 3, 4, 5 and 6 mL were pipetted out into different conical flasks and the volume was made up to 10 mL by adding required volume of potassium dichromate solution. Then, to each conical flask, concentrated sulphuric acid (20 mL) was added slowly. The resulting solution was allowed to stand for half an hour and distilled water (10 mL) was added and left overnight. The intensities of the colours developed in different conical flasks were measured next day with the help of colorimeter by selecting filter at 660 nm. The graph of concentration against absorption was plotted to get the standard curve.

Soil sample (15 g) was ground and sieved through 100 mesh sieve. Then, 1g of this sample was transferred to a conical flask (100 mL). To this, standard potassium dichromate solution (10 mL) and concentrated sulphuric acid (20 mL) were added.
slowly. After half an hour, this solution was diluted by adding distilled water (10 mL). It was left overnight and the intensity of the colour was measured by using colorimeter.

With the help of this reading, using standard curve, the percentage of organic carbon present in the soil was calculated using the following equation,

\[
\text{Percentage of organic carbon} = \text{Graph reading} \times 0.042 \quad (3)
\]

\[
= 18.3 \times 0.042
\]

\[
= 0.7
\]

The percentage of carbon in soil sample was found to be 0.77% and corresponding nitrogen content was 0.077%. This result indicates that the soil was less fertile with respect to organic carbon and nitrogen.

3.2.3 Determination of available phosphorus (Kg/acre)

Molybdenum blue method was used for accurate determination of available phosphorus.

Generally, available phosphorus in the soil sample is extracted in the solution form. For this purpose mainly two extractants are commonly used.

- Olsen extractant.
- Bray’s extractant.

The proper extract was selected on the basis of the nature of the soil. Olsen extractant is used for neutral and alkaline soils, whereas Bray’s extractant is used for acidic soils. pH value of the soil suggests Bray’s extractant to be used to determine the available phosphorus in the solution form.

It was estimated by converting the dissolved phosphorus into phosphor molybdate complex. This complex was reduced by using standard solution of ascorbic acid. The reduced compound has blue colour, the intensity of which depends upon the
concentration of phosphorus present in the solution. The intensity of the colour was measured colorimetrically.

\[ \text{H}_3\text{PO}_4 + 12 \text{H}_3\text{MnO}_4 \rightarrow \text{H}_3\text{P} \left[\text{Mo}_3\text{O}_{10}\right]_4 + 12 \text{H}_2\text{O} \]

- **Colorimetric method**

The determination of available phosphorus by colorimetric method, involved the following steps:

- **Preparation of Bray’s extractant**

- **Reagent A**

  Ammonium molybdate (12 g) was dissolved in distilled water (200 mL). Similarly potassium antimony tartarate (0.2908 g) was dissolved in distilled water (200 mL). These two solutions were mixed and to the mixture, sulphuric acid (5 N, 1000 mL) was added and the solution was made upto 2 liters by using distilled water. The solution was stored in cool and dark place.

- **Reagent B**

  It was prepared by dissolving ascorbic acid (1.056 g) in reagent A (200 mL). This solution was prepared just before the experiment.

- **Stock solution of phosphorus pentoxide**

  Ammonium dihydrogen orthophosphate (0.479 g) was dissolved in distilled water and the volume was made upto 1000 mL with distilled water. This solution had 250 ppm concentration of P$_2$O$_5$.

- **Working standard**

  The stock solution (20 mL) of P$_2$O$_5$ was diluted to 1000 mL by using distilled water, to reduce the concentration of P$_2$O$_5$ to 5.0 ppm.
• Standard curve

The working standard solution was accurately pipetted out 0, 2, 4, 6 and 10 ml to beakers (50 mL). To this solution, Bray’s extractant (5 ml) was added and the volume was made up to 20 mL by adding appropriate volume of distilled water. To the resulting solution, reagent B (4 mL) was added and kept aside for 15-20 minutes. The intensity of the colour was measured using colorimeter. The graph of concentration against absorption was plotted to obtain standard curve.

Soil sample (2.5 g) was taken and Bray’s extractant (50 mL) was added, shaken thoroughly and filtered. The filtrate (5 mL) was taken in a separate conical flask and to this, sulphuric acid (5N, 0.5 mL) and distilled water (16 mL) were added. To this solution reagent B (4 mL) was added, stirred well and allowed to stand for 15-20 minutes. The intensity of the colour developed was measured colorimetrically. The concentration of available phosphorus present in the soil was determined by using standard curve as follows,

\[
\text{Average } P_2O_5 \text{ Kg/acre} = A \times 3.6 \quad (4)
\]

Where ‘A’ is graph reading = 3.45

Thus, the amount of phosphorus in soil sample under examination was found to be 12.45. This value indicates that the soil is less fertile with respect to available phosphorus.

3.2.4 Determination of potash content (Kg/acre) in the soil sample

Mineral soils generally contain potassium in larger quantities than nitrogen and phosphorus. The technique of Flame Emission Spectroscopy (FES), formerly called
flame photometry, was employed for the determination of potassium present in the soil in terms of available potassium oxide (in kg/acre).

- **Principle of FES**

  In this technique, a solution containing a metallic salt is aspirated into the flame. The vapours now contain atoms of the metal. Some of these gaseous metal atoms absorb energy and raised to higher energy level to permit emission of radiation characteristic of the metal. The intensity of the radiation can be measured by using photoelectric detectors. The experiment is carried out in the following steps.

- **Preparation of standard solution**

  The standard solution of 1000 ppm potassium oxide was prepared by dissolving potassium chloride (1.583 g) in distilled water (1000 mL). From this stock solution, 10, 20, 30 and 40 ppm K₂O solutions were prepared by diluting 10, 20, 30 and 40 mL of stock solution with neutral ammonium acetate solution (1N, 1000 mL). The neutral ammonium acetate solution helps to extract potassium present in the soil sample in solution form.

- **Procedure**

  The standard solutions having different concentration (1.0 mL each) was aspirated into the flame and intensities of the radiation was measured by using flame emission spectrometer. The standard curve was obtained by drawing a graph of concentration (known solution) against intensity of radiation and the similar procedure was employed for soil sample.

  Soil sample (10g) was ground and neutral ammonium acetate solution (1 N, 25 mL) was added and the mixture was shaken thoroughly to get phosphorus present in the soil sample to solution form. Then, the solution was filtered and 0.5 mL of this solution was aspirated into the flame in the instrument. The intensity of radiation was
recorded and compared with standard curve. The amount of potassium present in the soil was determined by using the following Equation,

\[
\text{Available K}_2\text{O (Kg/acre)} = \frac{A}{10^6} \times \frac{\text{Volume of the extractant}}{\text{Weight of the sample}} \times \frac{2 \times 10^6}{2.2}
\]

\[
\text{Available K}_2\text{O (Kg/acre)} = \frac{A}{10^6} \times \frac{25}{10} \times \frac{2 \times 10^6}{2.2}
\]

\[= A \times 2.27 \quad \text{(5)}\]

Where, ‘A’ is the concentration of K\(_2\text{O}\) in ppm.

Therefore, available K\(_2\text{O}\) (kg/acre) = A \times 2.27

\[= 44.44 \times 2.27 \]

\[= 63.12\]

Therefore, available K\(_2\text{O}\) (Kg/acre) = 101

Therefore, from this value, it can be concluded that the soil under examination was found to be moderately fertile in nature with respect to potash.

3.2.5 Determination of micronutrients

Sixteen elements are essential for plants for their normal growth and development. Out of these, seven are classified as micro nutrients since their requirement is in small amounts. They are Zn, Cu, Mo, Cl, Mn, B and Fe. These nutrients are present in the soil in two forms, i.e., in available and unavailable forms.

The concentration of micro nutrients like Fe, Cu, Zn and Mn was determined by using Atomic Absorption Spectrophotometer (AAS).

Procedure

Soil sample (10 gm) was mixed with DTPA (diethylene triamine penta acetic acid) solution (20 mL). The mixture was stirred mechanically for 2 hr. The solution was filtered and the filtrate (0.5 mL) was aspirated into the flame of AAS and the
intensity of radiation was recorded for Zn, Cu, Mn and Fe using appropriate filters. By using this intensity of radiation, the concentration of these metals was determined with the help of standard curves. The exact amount of these micro nutrients was calculated by using the following equation,

\[
\text{Available micronutrients} = \frac{\text{Xppm}}{10^6} \times \frac{\text{Volume of the extractant}}{\text{Weight of the sample}} \times 10^6
\]

\[
\text{Available K}_2\text{O (Kg/acre)} = \frac{\text{Xppm}}{10^6} \times \frac{20}{10} \times \frac{10^6}{10}
\]

\[= X \times 2 \quad \text{(6)}\]

Thus, (i) available Zn in the soil \(= 0.29 \times 2 \) = 0.58 ppm

(ii) available Cu in the soil \(= 0.24 \times 2 \) = 0.48 ppm

(iii) available Mn in the soil \(= 2.35 \times 2 \) = 4.70 ppm

(iv) available Fe in the soil \(= 4.10 \times 2 \) = 8.2 ppm

Therefore, the values obtained above indicate that the soil sample under examination was moderately fertile with respect to available Zn, Cu, Mn and Fe. The results are tabulated in table 1.

Table- 1. Summary of the Soil Analysis

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Examined Parameters</th>
<th>Unit</th>
<th>Report</th>
<th>Nature of the Soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>-</td>
<td>6.2</td>
<td>Less acidic</td>
</tr>
<tr>
<td>2</td>
<td>Organic Carbon</td>
<td>%</td>
<td>0.77</td>
<td>Less fertile</td>
</tr>
<tr>
<td>3</td>
<td>Organic Nitrogen</td>
<td>%</td>
<td>0.077</td>
<td>Less fertile</td>
</tr>
<tr>
<td>4</td>
<td>Available Phosphorus</td>
<td>Kg/acre</td>
<td>12.45</td>
<td>Less fertile</td>
</tr>
<tr>
<td>5</td>
<td>Available Potash</td>
<td>Kg/acre</td>
<td>101</td>
<td>Moderately fertile</td>
</tr>
<tr>
<td>6</td>
<td>Available Zinc</td>
<td>ppm</td>
<td>0.58</td>
<td>Moderately fertile</td>
</tr>
<tr>
<td>7</td>
<td>Available Cu</td>
<td>ppm</td>
<td>0.48</td>
<td>Moderately fertile</td>
</tr>
<tr>
<td>8</td>
<td>Available Mn</td>
<td>ppm</td>
<td>4.70</td>
<td>Moderately fertile</td>
</tr>
<tr>
<td>9</td>
<td>Available Fe</td>
<td>ppm</td>
<td>8.20</td>
<td>Moderately fertile</td>
</tr>
</tbody>
</table>
Data of soil analysis suggests that the soil where the plant was collected is slightly acidic, less fertile with respect to organic carbon and nitrogen, moderately fertile with respect to P, K, Zn, Cu, Mn and Fe.

References


