

# **REVIEW OF RESEARCH**

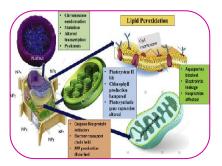


## PHYSICO-CHEMICAL PROPERTIES OF RHIZOSPHERIC SOILS

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#### ABSTRACT

The rhizospheric soils from eleven selected sites were collected on seasonal bases along with the available native host over a period of one year. At every selected site, the collection method employed was the same, i.e. rhizospheric soil cores from 5-10 different sites around roots of host plants were collected. The collected soil/ rhizospheric soil cores were mixed thoroughly and were sub-sampled by repeated quartering and pooling opposite quarters (Ambler and Young, 1977). Fine root system of the native host species was also separated and collected for analysis of mycorrhizal status. The host as well as the rhizospheric soil



were packed in labeled polythene bags separately and were brought to the laboratory of Shankarrao Mohite College, Akluj.

The rhizospheric soil collected during the three seasons i.e. rainy, winter and summer season from all the eleven sites after bringing to the laboratory were allowed to air dry. These soil samples on air drying were then sieved through sieves of holes of 2 mm diameter and stored in labeled airtight plastic containers at  $4^{\circ}C$  for further physico-chemical analysis and spore extraction.

**KEYWORDS**: physico-chemical, Electrical conductivity, rhizospheric soil

## **INTRODUCTION**

The physico-chemical properties of the rhizospheric soils collected were evaluated using the standard methods described in (Jackson, 1967). The physico-chemical properties that were studied as follows:

#### a. Soil temperature:

The soil temperatures of the selected eleven sites were taken per season during every field collection. The soil temperature was recorded with the help of a digital soil thermometer. The stainless-steel probe of the instrument was inserted 6" deep in the upper earth crust and the temperature was recorded.

### b. Soil moisture:

The soil samples on reaching the laboratory were immediately put into separate porcelain dishes and weighed ( $W_I$ ) as initial weight. The porcelain dishes were then placed inside preheated oven at 105°C. The porcelain dishes were removed from the oven after 2 hours and final weight ( $W_F$ ) was recorded

separately for each soil sample. The difference of the two weights i.e. initial weight ( $W_1$ ) and final weight ( $W_F$ ) were recorded for each soil samples as soil moisture content or loss in weight on drying and were converted into percent soil moisture content by using following formula:

Soil Moisture % = Loss in weight of sample on drying x 100 Initial weight of sample

#### c. Soil pH:

The soil pH was calibrated using a digital pen pH meter (Hanna Instruments) as soon as the samples reached the laboratory. The eleven different soil samples were made into aqueous slurry separately in ten different 100 ml beakers by dissolving 10gm (1:10 w/v) of soil sample in 100 ml of distilled water. The aqueous slurries were stirred with clean glass rod and were left undisturbed for 30 minutes. The pH of the clear aqueous solutions was then calibrated and recorded separately.

#### d. Electrical conductivity:

The above-prepared aqueous solutions were again immediately used to read the electrical conductivity of the respective soil samples. The electrical conductivity was calibrated with the help of a conductivity pen (TDScan 20 of Eutech Cybernetics).

#### e. Soil texture:

In the International system of description of soil particles, soil is graded as Coarse Sand (2 mm particle size), Fine Sand (0.2 mm particle size), Silt (0.02 mm particle size) and Clay (0.002 mm) based on soil particle size.

It was therefore, sieves of 2 mm ( $S_1$ ), 0.2 mm ( $S_2$ ), 0.02 mm ( $S_3$ ) and 0.002 mm ( $S_4$ ) were placed one above other in a vertical tire of descending order. Each air-dried soil sample was weighed ( $W_1$ ) to 100 gm and poured on the topmost sieve. The sieve tier was shaken so as to allow the soil particle of different size to pass through different sieves. The sediment on each sieve was weighed and recorded as  $W_1$  (weight of soil on  $S_1$ ),  $W_2$  (weight of soil on  $S_2$ ),  $W_3$  (weight of soil on  $S_3$ ) and  $W_4$  (weight of soil on  $S_5$ ) respectively. The soil particles of different grades were calculated in percent.

#### f. Organic Carbon - Walkley and Black method (Jackson, 1967):

**Reagents:** 1N potassium dichromate solution (49.04gm per litre). 0.5N ferrous ammonium sulphate (196gm of the hydrated salt per litre containing 20ml  $H_2SO_4$ ). Diphenylamine indicator (0.5gm diphenylamine dissolved in 20ml of water and 100ml of conc.  $H_2SO_4$ ) mixture. Concentrated  $H_2SO_4$  (AR) containing 1.25% silver sulphate, Ortho-phosphoric acid.

**Procedure:** 1gm of air-dried soil (ground and sieved through 0.2mm) was placed at the bottom of a dry 500ml conical flask (Borosil). 10ml of 1N potassium dichromate was pipetted in the flask which was kept on an asbestos sheet. Then 20ml of  $H_2SO_4$  (containing 1.25% silver sulphate) was added and swirled again two or three times. The flask was allowed to stand undisturbed for 30 minutes. After half an hour, 200ml of distilled water was added. After the incorporation of 10ml phosphoric acid to the flask, 1ml of diphenylamine was added as a indicator and the content of the flask was titrated with 0.5N ferrous ammonium sulphate solution, till the blue-violet colour changes to green. A blank without soil was run before and after the completion of actual titration of soil solutions.

Calculation: The organic matter in percentage was calculated by using following formulae:

Organic carbon (%) = 
$$\frac{10(B-T)}{B} \times 0.003 \times \frac{100}{wt.ofsoil}$$

Where: B: Volume of ferrous ammonium sulphate required for blank.

T: Volume of ferrous ammonium sulphate solution consumed for soil sample.

# g. Mineralizable Nitrogen (Jackson, 1967):

**Reagents:** 0.32% potassium permanganate solution, 2.5% sodium hydroxide, liquid paraffin, 0.1N  $H_2SO_4$ , 0.1N NaOH solution and methyl red indicator (0.066gm methyl red, dissolved in 100ml of 95% alcohol).

**Procedure:** Digestion: 5gm of air-dried soil was taken in 500ml Kjeldahl flask. 5ml of water was added, followed by addition of 15ml of concentrated H<sub>2</sub>SO<sub>4</sub>. The setup was kept undisturbed for 30 minutes. To this mixture was added 0.1gm of selenium powder. The digestion was first started over small flames and gradually increased until white fumes of sulphuric acid were produced. The flask was removed immediately and added 5gm of potassium sulphate. The flask was again replaced over the flame and digestion was continued for 1 to 1½ hours till the digest became colourless. The flask was allowed to cool and then 50ml of water was added. The solution was left for 30 minutes to allow the soil particles to settle down. With the help of pipette, the top layer of clear soil extract was filtered out and stored in plastic bottles.

**Distillation:** From the stock, 10ml of soil extract was poured into a 500ml distillation flask. This was diluted by 100ml of water and also 10% NaOH was added to make the mixture in the digestion flask neutral. The mixture was well shaken and distillation commenced. The liberated ammonia was collected in 25ml of 0.1N HCl containing two to three drops of methyl red indicator. The distillation was carried out until the distillate was about 1/3<sup>rd</sup> of the liquid has passed over. When the distillation was over, the condenser tube was rinsed with distilled water to remove any traces of nitrogen if trapped into the 0.1N HCl.

A blank without soil extract was carried out in exactly the same manner.

The percentage of nitrogen in the soil was calculated on the bases of 5gm soil by using the following formula:

Nitrogen % = (B-T) x N x 
$$\frac{0.14}{wt.ofsoil}$$
 x 100

0.14

= (B-T) x 0.1 x  $0.5^{-1}$  x 100 = (B-T) x 2.8 x 10<sup>-3</sup> x100 = (B-T) x 0.28

Where:

B= Blank titration (i.e. ml of alkali used).

T= Actual titration.

N= Normality of the standard alkali.

#### h. Available Potassium- (Jackson, 1967):

**Reagents:** Neutral normal ammonium acetate [Equal volume of 2N acetic acid and 2N ammonium hydroxide were mixed and the pH adjusted to 7 with acetic acid. 125gm of ammonium acetate in 1.6lit of distilled water (1N)], Potassium chloride (AR).

**Preparation of standard potassium curve:** A stock solution of 100 ppm of potassium was prepared by dissolving 1.9084gm of potassium chloride (AR grade and dried at 60°C for 1 hour) in distilled water and final volume of the stock made to 1000ml. From this stock, 10 to 40 ppm of potassium was diluted with the ammonium acetate solution. After attaching the appropriate filter (404 nm) for potassium and adjusting the gas and air pressure, the flame photometer reading was set at 100 for 40 ppm potassium. The curve was obtained by plotting the readings of the flame photometer against the different concentrations i.e. 10, 15, 20, 25, 30, 35 ppm of potassium.

**Procedure:** 5gm of air-dried soil was shaken with 25ml of neutral 2N ammonium acetate for 5 minutes and allowed to stand still for 30 minutes. After 30 minutes, the content was filtered immediately through a dry filter paper. The filtrate was moved to the flame, photometer and meter reading corresponding to the soil extract was noted. Percentage of potassium was calculated by using following:

# = R x 11.2

where: R = ppm of potassium in extract calculated from standard curve. Therefore,

% of K =  $ppm of K \times 100$ 1000

# i. Available Phosphorus - (Jackson, 1967):

**Reagents:** 

- 1. 0.5 M NaHCO<sub>3</sub> solution pH 8.5: 42g NaHCO<sub>3</sub> in 1 ltr adjust pH with 0.1 N NaOH.
- 2. Activated charcoal Darco G-60.
- 3. Solution A:

i.Dissolved 12 g ammonium molybdate in 250-300 ml distilled water.

ii.Dissolved 0.291 g antimony Potassium tartrate in 100 ml distilled water.

iii.Prepared 1000 ml of 5N H<sub>2</sub>SO<sub>4</sub> (140 ml conc. H<sub>2</sub>SO<sub>4</sub> in one litre).

Cooled ammonium molybdate (solution 'A') was added to  $5N H_2SO_4$  (solution 'c') followed by cooled antimony potassium tartrate (solution 'b') volume was made upto 2 litres. (store in a brown bottle in a cool place).

4. **Solution B**: Dissolved 1.056 g Ascorbic acid (LR) in 200 ml of solution 'A'. (Solution B was prepared fresh as and when required).

5. 0.25 % to 2,4: P Nitrophenol and 5N H<sub>2</sub>SO<sub>4</sub> to adjust pH (5.0) using KH<sub>2</sub>PO<sub>4</sub>.

6. **Standard P solution**: 0.439 g AR grade  $KH_2PO_4$  dried in an oven at 60 °C for 1 hr and cooled in a desiccator and dissolved in 500 ml distilled water + 25 ml 7N  $H_2SO_4$  (approx), volume made up to one litre. This gave 100 ppm stock solution of P (100 micrograms per ml).

7. Working P solution: Taken 10 ml stock solution (100 ppm P) in a 500 ml volumetric flask make up the volume. This is a 2 ppm solution of P (2 micrograms per ml.)

# Procedure:

# **Extraction:**

- 1. Take 2.5 g soil in 100 ml conical flask.
- 2. Added to it, a little activated charcoal Darco-G 60 followed by 50 ml of 0.5 M NaHCO3 solution.
- 3. Corked the flask and shook it for exactly 30 minutes on a platform type shaker.
- 4. Filtered the contents immediately after shaking and collected the filtrate.

## **Estimation:**

1. Pipetted 5 ml of the NaHCO<sub>3</sub> extract into a 25 ml volumetric flask.

2. Added two drops 2,4-p nitrophenol and then added 5N  $H_2SO_4$  drop by drop with intermittent shaking till the yellow colour disappears.

3. The content was diluted to about 20.0 ml with distilled water and then added 4.0 ml. Solution B (Ascorbic acid).

4. The volume was made up and the intensity was measured in blue colour at 730 to 840 nm on the spectrophotometer.

# **Preparation of standard curve:**

1. Prepared a series of standards by taking 0, 1, 2,3,4,5 and 10ml 2 ppm P solution in 25 ml vol. flasks separately.

- 2. Added 5 ml of NaHCO<sub>3</sub> solution to each flask and adjusted the pH as above.
- 3. Developed colour and recorded the reading as above.
- 4. A graph was plotted, readings on X axis and conc. of P on Y axis.

#### **Calculations:**

Available phosphorus (%) = 
$$R \times \frac{\text{Total Vol. of extract}}{\text{Vol. of aliquot taken}} \times \frac{1}{\text{Wt. of soil}} \times 10$$

Where, R = Graph P value (Find out value of R from the std. curve)

# j. Soil microelement extraction - DTPA Test (Lindsay and Norvell, 1978):

1. **DTPA Extracting solution**: It was made from 0.005M DTPA (diethylene triamine penta acetic acid), 0.01M CaCl<sub>2</sub>. 2H<sub>2</sub>O, 0.01M Triethanolamine (TEA) with pH adjusted to 7.3.

2. **Extraction:** Ten gm of air-dried soil sample was placed in 125 ml conical flask. Add 20ml of the DTPA extracting solution. The flasks were covered with plastic sheets and secured tight on a horizontal shaker. The flasks were shaken for 2 hours. Suspension was then filtered by gravity through Whatman No. 42 filter paper. Filtrate was then analysed for microelements using inductively coupled plasma atomic emission spectroscopy at Soil Testing Laboratory, Dept. of Agriculture, Govt. of Maharashtra, Pune and by using appropriate standards of each element. The microelements analysed were iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn).

# **CONCLUSION**

The difference of the two weights i.e. initial weight and final weight were recorded for each soil samples as soil moisture content or loss in weight on drying and were converted into percent soil moisture content by using following formula:

The eleven different soil samples were made into aqueous slurry separately in ten different 100 ml beakers by dissolving 10gm of soil sample in 100 ml of distilled water.

In the International system of description of soil particles, soil is graded as Coarse Sand , Fine Sand , Silt and Clay based on soil particle size.

A blank without soil was run before and after the completion of actual titration of soil solutions.

Preparation of standard potassium curve: A stock solution of 100 ppm of potassium was prepared by dissolving 1.9084gm of potassium chloride in distilled water and final volume of the stock made to 1000ml.

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