STUDENT STUDY PROJECT ON

Macro Nutrients Analysis to Determine Soil Fertility

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CERTIFICATE

This is to certify that students of the B. Sc –MICROBIOLOGY – Second year has been successfully completed the project entitled "**Macro Nutrients Analysis to Determine Soil Fertility"** from the department of Microbiology ,Kakatiya government college, Hanamkonda.

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TITLE: - Macro Nutrients Analysis to Determine Soil Fertility

1. Hypothesis:

Soil is the critical component of the earth system functioning for the production of food, fodder, fiber and also maintains environment quality. It is the basic natural medium for the plants, and diverse micro and macro flora and fauna. Soil nature and its fertility depend on the types and interaction of micro and macro fauna and flora it harbors, which in turn influence the plant nutrition. The vital plant nutrients are Nitrogen, Phosphorous, and Potassium, which are also called as Essential nutrients. Now a days decreasing crop yields and food nutrients in the crops is due to poor soil quality or lack of vital / essential nutrients in the soil.

As the essential nutrients are needed to the efficient plant growth and crop yield, the project focused on detailed study of physical and chemical properties of representative soil samples from selected villages in Khammam rural region. Fertility status of the soil was determined by interpreting the results obtained by the above study. Interpretation of soil chemical status involves an estimation of its available nutrient status (George rehm et al., 2002).

2. Aims and Objectives

2.1.AIM:

To determine the soil fertility status of selected region by estimating the soil available nitrogen (N), available phosphorus (P) & available potassium (K) levels and physical characteristic features.

2.2.Objectives:

- ✓ To study the soil physical characters like Texture, color and moisture etc.
- ✓ To evaluate Soil pH, Electric Conductivity and organic carbon.
- ✓ To estimate the soil macro nutrients available i.e., available Nitrogen, available Phosphorous and available Potassium.
- ✓ To determine the soil fertility using above observations.
- ✓ To understand the importance of soil testing before crop practice.
- ✓ To know about the nature of soil in the selected sites of study i.e Khammam rural villages.

3. Review of Literature:

Soil characterization in relation to evaluation of fertility status of soils of an area or region is an important aspect in context of sustainable agricultural production. Nitrogen, phosphorus, potassium, sulphur, boron and zinc are important soil elements that control it's fertility and yields of the crops. T. Sujatha *et al.*, (2013). The structure of the soil microbial community is an important component of soil quality and health. Soil microbiological properties could be early and sensitive indicators of anthropogenic effects on soil ecology in both natural and agricultural ecosystems. Suzanne Visser *et al*., (2009).

Soil is very important and a valuable resource for every human being. Soil is the mixture of rock debris and organic materials, which develop on the earth's surface. The major factors

that determine soils' characteristics are parent material, climate, relief, vegetation, time, and some other life-forms. Major constituents of the soil are mineral particles, humus, water, and air. A soil horizon is a layer generally parallel to the soil crust, whose physical characteristics differ from the layers above and beneath. Anderson, J.L., et al (2001)

Soils were classified on the basis of their inherent characteristics and external features including texture, color, slope of land, and moisture content in the soil.Soil Survey of India, established in 1956, made comprehensive study of soils. Aubert, G *et al* (1972) .On the basis of genesis, color, composition, and location, the soils of India have been classified as: Alluvial soils ,Black soils ,Red and Yellow soils ,Laterite soils ,Arid soils ,Forest soils Saline soils and Peaty soils Cottenie, A.,*et al* (1981)

Alluvial soils are widespread in the northern plains and the river valleys and cover about 40% of total area of India. Alluvial soils are depositional soils, as transported and deposited by the rivers streams. Alluvial soils are normally rich in potash, but poor in phosphorous.

In the Upper and Middle Ganga plain, two different types of alluvial soils are found i.e. *Khadar* (it is the new alluvium and is deposited by floods annually) and *Bhangar* (it is a system of older alluvium, deposited away from the flood plains). The alluvial soils normally vary in nature from sandy, loamy, to clayey and its color varies from light grey to ash grey .

Also popular as Regur Soil or the Black Cotton Soil, Black soil covers most of the Deccan Plateau; for example, black soil is found in parts of Maharashtra, Madhya Pradesh, Gujarat, Andhra Pradesh, and Tamil Nadu. Black soil is usually clayey, deep, and impermeable; therefore, it can retain the moisture for a very long time (very useful for the crops especially cotton). Black soil is rich in lime, iron, magnesia, alumina, and also potash. *Bellotto, M., et al., (2014)*

Red soil develops on crystalline igneous rocks in the areas of low rainfall, especially, in the eastern and southern parts of the Deccan Plateau. Red soil develops a reddish color because of a wide diffusion of iron in crystalline and metamorphic rocks. On the other hand, it develops yellow color when it occurs in a hydrated form. The fine-grained red and yellow soils are usually fertile, whereas coarse-grained soils found in dry upland areas have poor fertility. Kang, B.T. *et al (1986).*

The fourth criterion is used because some specific plants need certain elements. For example, cobalt (Co) is required by bacteria responsible for nitrogen (N) fixation in legumes; therefore, Co is classified as 'beneficial', rather than essential. Silica (Si) is not 'essential', but highly 'beneficial' to help plants cope with multiple stresses. Other beneficial elements include sodium (Na) and vanadium (V).

Plants require 17 nutrients, also called 'essential elements', which assist with different plant functions for growth and reproduction. Each plant nutrient is needed in different amounts and varies in how mobile it is within the plant and the soil. It is useful to know the relative amount of each nutrient that is needed by a crop in making fertilizer recommendations. In addition, understanding plant functions and mobility within the plant are useful in diagnosing nutrient deficiencies. Soil characteristics that affect nutrient availability to plants are also presented, as they influence nutrient management decisions. Clain Jones *et al.,* (2016).

4. Research Methodology

4.1.Soil sampling:

The selected areas for soil collected are the villages named Kamanchikallu, Peddamanduva, Kamalapuram, Ammapeta, Theerdhala, pallegudem, Pandurangapuram, Danavayagudem located near Khammam and Mudigonda mandal of Khammam District.They are mostly rain fed and few are irrigated by bore wells. The representative soil samples were collected in duplicate and analysed. Each site has given numbers like sample-1,2,3…16.

4.1.1.Collection of representative soil sample:

Depending upon of the field condition and the objective of the samplings, we mostly used spade. Based on different soil types, colour, crop growth or the slop, the area is divided into different homogenous units. Brady *et al,*. (2006).The uniform field was clearly demarcated with specific sampling points by the zig –zag fashion or randomly in such a way that whole field was covered for the sampling. At the sampling site the extra fertile layer and the surface liter was removed using spade ,then using anger sample was collected in a plastic bowl and transferred to bags. During the sampling, the soil was found hard, then a 'v' shaped cut was done into the soil at a depth of 15 cm then the soil in the pit was removed.

The collected soil samples were stored in the polythene bags of 6x 8 sizes, made up of a film about negligible thickness, which were sealed by furisting; some were by tying the neck by means of rubber bands or adhesives tape. The collected soil was used for the estimation of macronutrients like nitrogen, phosphorus, potassium so use of metallic tools was highly avoided seriously fried to use stick [or] stainless steel.

The soil sample was collected and information was furnished as below.

- Sample number
- Name of address of the farmed
- Details of field
- Date of sampling
- Number of crops grown
- Name of crop growing in this season
- Sources of irrigation
- Type of fertilizer using ,either chemical [or] bio-fertilizer

- ❖ Date \month of harvesting of the previous crop
- ❖ Any technical [or] seasonal problem observed in the crop

5.1.1.1.Sample preparation for testing.

- ✓ Spreader sample for drying on clean cloth, plastic [or] brown paper sheet.
- ✓ Removed the stone pieces, roots, leaves &other un-decomposed organic residues from the samples.
- ✓ Large lumps or moist soils should be broken.
- ✓ After air drying these samples have been crushed gently and sieved through a nylon sieve.
- ✓ About 250g of sieved sample was used and labeled in the sample bag for testing.

Precautions taken during the soil sample collection.

- ✓ The ideal and preferred time for soil sampling is just after the harvest of the rabi crop
- ✓ Removed all debris from the surface before collection of soil samples.
- ✓ Avoided taking of the samples from upland and low land areas in the same field
- ✓ Taken separate samples from the areas of the different appearance.
- ✓ In row crop taken samples in between the rows.
- ✓ Kept the samples in a moisture free clean bag.
- ✓ Samples were taken in a small area less than 1-2 hectares.
- ✓ For the analysis rust free spade and Kurpi were used and kept in clean polythene bags.
- ✓ Samplings was not done nearer to the trees and from the place where fertilizers and manure were not used for storing the chemical, fertilizers were placed.
- ✓ Clean bags were used for sample collection .bags used for storing the chemical; fertilizers and manure were not used for sample holding.

4.1.2.Storing Soil samples:-

- ✓ The register and labeled samples in laboratory are finally placed in a cardboard carton. Label the carton properly with the details of soil sample and stored in the separate room.
- ✓ The room was kept away from direct sunlight/wind.

4.2. ELEMENTAL ANALYSIS

4.2.1: Estimation of available Nitrogen:

Reagents:

- 0.32% potassium permanganate ($KMnO_4$) solution.
- 2.5%sodium hydroxide (NaOH).
- 2%boric acid solution containing 20-25ml of mixed indicator / liter.
- Mixed indicator: 0.066g methyl red + 0.99g bromocerol green dissolve in 100 ml of 95%alcohol.
- 0.02 N sulphuric acids (H_2SO_4).

Procedure:-

i. Weigh 5 g of prepared soil sample and transfer it to the digestion tube.
ii. Load the tube in distillation unit and other sides of those keep 20 ml of 2%boric acid with mixed indicator in 250 ml conical flask.
iii. 25 ml each of potassium permanganate (0.32%) and sodium hydroxide (2.5%) solution is automatically added by distillation unit programmer.
iv. The sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 20ml of 2% boric acid containing mixed indicator solution kept in a 250 ml conical flask.
v. With the absorption of ammonia, the pinkish colour turns to green.
vi. Nearly 150 ml of distillate is collected in about 10 minutes.
vii. The green colour distillate is titrating with 0.02N sulphuric acid and the colour changes to original shade (pinkish color).
viii. Simultaneously, blank sample (without soil) is to be run.
ix. Note the blank & sample titer reading (ml) and calculate the available nitrogen in soil.

4.2.2: Estimation of available Phosphorous

A) *Olsen's method for the neutral & alkaline soils*

Principle

The most widely used extranctant is the 0.5M NaHCO3 solution at the pH 8.5. the reagent is most widely suitable for neutral to alkaline soils and is designed to control the ionic activity of calcium through solubility product of $CaCO_3$ thus extracting the most reactive forms of P from Al- , Fe-, and Ca- phosphates. The solubility calcium phosphate is increased because of the precipitation of the Ca^{++} as $CaCO_3$. Phosphorous is the extract can be determined using suitable method of colour development and measuring the color intensity at an appropriate wave length.

Instruments

Colorimeter or spectrophotometer, mechanical shaker for Extraction of the available phosphorous

It is prepared by the dissolving of 42.0 g of NaHCO3 (laboratory reagent) in distilled water to give on liter of the solution. The pH is adjusted to the 8.5 with the small quantities of the 10% NaOH.

PROCEDURE

- Take 2.5 g of soil in 100ml conical flask, add a liter of Dacron G 60 charcoal powder (free of phosphorous) followed by 50ml of Olsen's reagent.
- Run the blank with the soil
- Shake the flask for 30 minutes on the platform type shaker and filter the contents immediately through the dry filter paper (what man paper no.1) into a clean and dry beaker or vial.
- Estimate the phosphorous calorimetrically by atonable and Olsen's procedure.

CALICULATION

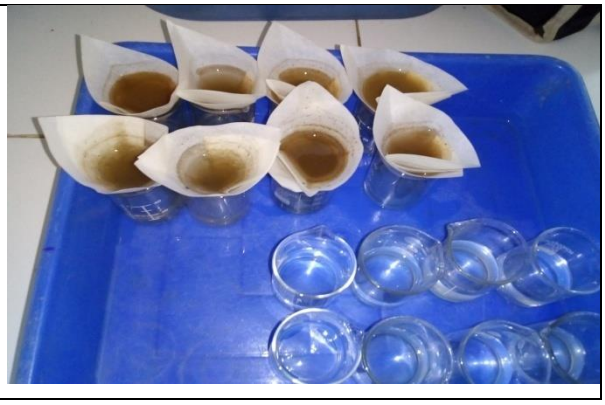
$$(Available\ P2O5\ (OR) OLSEN'S P2O5\ (kg/ha)) = R \times \frac{total\ volume\ of\ the\ extract}{weight\ of\ the\ soil\ taken} \times \frac{25}{volume\ of\ the\ aliquot} \times \frac{2.24}{1} \times 2.29$$

Where,

R= ppm of P in the aliquot (to be seen from the standard curve)

$$\left(Available\ P2O5\ \left(\frac{kg}{ha}\right)\right) = R \times \frac{50}{2.5} \times \frac{25}{5} \times \frac{2.24}{1} \times 2.29 = R \times 513$$

$$\left(Available\ P2O5\ \left(\frac{kg}{acre}\right)\right) = R \times 208$$



5.2.3: Estimation of available Potassium:

The available potassium exchangeable and water soluble potassium is determined by extracting soil with neutral normal ammonium acetate solution. The estimation of potassium is carried out by flame photometer.

Principle:

The principle underlying this is that a large number of elements when excited in a flame, emit radiation of characteristic wave length. The excitation cause one of the outer electron of neutral atoms to move to an outer orbit of higher energy level or the atoms may be excited sufficiently to lose an electron completely from the attractive force of the nucleus where excited atom return to the lower energy, light at characteristics is emitted. Excited atom or ions give line radiation at very definite wave length and thus K gives at 404.4 and 767(mu). The flame photometer employs relatively low temperature excitations and a measure with a photocell the emission intensity which is proportional and the concentrated in selected wave length (767 mu) and for these red filter is used.

Apparatus and reagent:-

A) Flame photometer with red filter.

B) Pipette, volumetric flask and conical flask

Reagent:-

(a) Natural normal ammonium acetate:

Add 58ml of glacial acetic acid to about 600 ml of H2o and then add 70ml of concentrated ammonia dilute the solution to one liter. Then adjust PH Of solution at 7.0 with the help of ammonia or acetic acid or this can be prepared amino. Acetic directly in H2o volume to be made ones then adjust the pH. 7.0.

(b) Stranded potation solution:

Dissolve 1.9066 gm of dried KCl in distilled water dilute to one liter. This 1000mg kg /1k solution. 100ml solution distilled to one liter to make 100ppm K solution.

Preparation of stranded curve:-

Take 0,1,2,3,4,5,6,7,8,9 and 10ml of 100mgkg/1 K solution different 25ml of volumetric flask. Make of the volume with 1N NH4O AC solution. Adjust the flame photometric reading the zero with the blank solution and at the 100 for 40mg kg /1K solution. Take the flame photometric reading for every dilution .plot the standard curve on the graph paper by taking K CONS. On X-axis and photometric reading on the Y-axis. This will give a factor (F) of the one flame photometric reading =0.4mgkg/k.

Procedure:-

Take 5gm of soil in 100ml of conical flask and add 25ml of 1N NH4O AC solution shake the content for the 5mints and the filter to through thewhat man no1 filter paper. Potassium extract is measured by flame photometer of caliber.

Precaution:-

- ✓ These should not be any turbidity or suspended practical is extract, it will chock the capillary feeding tube.
- ✓ The gas and air pressure should be constant.
- ✓ It sample reading goes beyond 100 then dilute the extract.

4.3. PHYSICAL ANALYSIS

4.3.1. Soil pH:

The pH value of a soil an indicator of soil reaction i.e. acidic, neutral or alkaline. The nutrient availability is governed by soil reaction. It is maximum at neutral pH and decreases with increase in acidity or alkalinity. Thus, pH value gives an idea about the availability of nutrients to plants.

- Principle:

The pH is usually measured by pH meter, in which the potential of hydrogen ion indicating electrode (glass electrode) is measured potentiometrically against calomel saturated reference electrode days, most of the pH meters have Single Combined Electorate. Before measuring the PH of the soil the instrument has to be calibrated with standard buffer solution of Known PH. Since, The PH is also affected by the temperature, Hence the PH meter should be adjusted to the temperature of the solution by temperature correction Knob

- Reagents:

Standard buffer solutions : these may be of PH 4.0,7.0 Or 9.2 And are prepared by dissolving one standard buffer standard buffer tablet in 100ml distilled water, it is necessary to prepare fresh buffer solution after few days .in absence of buffer tablet, a0.05 M potassium hydrogen Phthalate solution can be used which gives PH of a 4.0 (dissolve 10.21g. of A.R Greed potassium hydrogen Phthalate in distill Water and dilute to 1lt. Add 1 ml of chloroform or a crystal of thymol per liter as a preparative).

- Procedure:

(a) Soil to water ratio of 1:2 (PH2)

Take 20g of soil in 100ml beaker and add 40ml of distill water to it. The suspension is stirred at a regular interval for 30 mi. determine the PH by Immersing Elector` in suspension. For soils containing high salts, The PH should be determined by using 0.01M Calcium Chloride Solution. (Dissolve 0.110 grams of $CaCl_2$ in Water and dilute to 1lt)

(b) Saturate soil Paste (PHs)

Add small amount of distill to 250gr of Air dried soil. Stir the mixture with a spatula. At saturation the soil paste glistens and flows slightly when the container is tapped its slide freely and ensures cleanly of the spatula. After mixing allow the sample to stand for an hour if the paste as stiffened markedly or lost its glistening, add more water 0r if free water has collected on the surface of the paste, add an additional weighed quantity of dry soil and mixed it again. Then insert the electrode carefully in the paste and measure the PH.

(c) Saturation extracts (pH)

The soil is extracted using vacuum extractor and the PH is Measure in the saturation extractor.

Categories of soil pH values:

Soil PH		*Interpretation*
<5.0	:	Strongly Acidic
5.1-6.5	:	Slightly Acidic
6.6-7.5	:	Neutral
7.6-8.0	:	Mild Alkaline
>8.0	:	Strongly Alkaline

4.3.2. Determination of Electrical Conductivity:

Amount of Soluble Salts in a sample expressed in terms of the electrical conductivity and measure by a conductivity meter. The instrument consists of an AC sol bridge or electrical resistance bridge and conductivity cell having electrode coated with Platinum black. The instrument is also available as an already calibrated assembly (solubride) for representing the conductivity of solution in dSm^{-1} (Decisiemen per meter) 25^0 C.

Principle:

The simple wheat stone bridge circuit is used to measure EC by Null Method the bridge Consists of two Known and fixed resistance r_1, r_2, One Variable Standard resistance r_4 and the unknown r_3. The variable resistance r_4 is adjusted until a minimum or zero current flows Through the AC Galvanometer. At equilibrium

$$\frac{RI}{R2} = \frac{R3}{R4} \quad (OR) \quad R3 = \frac{R1}{R2} \times R4$$

Since Conductivity is reciprocal of receptivity, it is measured with the help of R_3

Reagents:

Potassium chloride: Dissolve 0.7456gr of dry potassium chloride (AR) in distills Water and make up the volume to 1lt

Procedure:

Take 20gr of soil in 100ml beaker, add 40m,l of distill water and shake intermittently for 30min. determined the conductivity of the supernatant liquid with the help of conductivity meter. The electrical conductivity of saturation extract (E.C.e) is also determent for salinity ratings.

Categories of soil ELECTRIC CONDUCTIVITY values:

ELECTRIC CONDUCTIVIT (dSm^{-1})	*EFFECT*
<1	- No deleterious effect on crop
1-2	- Critical for salt sensitive crops
2-3	- Critical for salt tolerant crops
>3	- Injurious to most crop

5. Results and Discussion

The representative soil samples 1 to 16 are analyzed and results were noted. All the representative values are the average of duplicate soil sample from each sites of collection.

5.1. Results

The soil type pH, Electric conductivity & Organic carbon are available macro nutrients,

Available' N', Available 'P' & Available' K' values were tabulated for analysis.

All the soils in the region are Black loam soils with neutral to moderate alkaline pH, and normal Electric conductivity. Organic carbon (OC%) is medium to high range which indicate available decomposing organic matter and moderate microbial activity.

Available macronutrients in each soil sample are determined and analyzed using standard methods. The observations are noted in table.1.

The results obtained were compared with the standard values according to methods manual, Department of Agriculture & Cooperation Ministry of Agriculture, Govt of India, New Delhi, 2011.Table.2.

Table:1. Values of Macro nutrients of soil samples.

Sample No	pH	E.C	OC (%)	N Kg/Acre	P Kg/ Acre	K Kg/Acre
1	6.46	0.08	0.4	147	03	76
2	7.93	0.14	0.4	120	11	47
3	7.97	0.24	0.75	182	05	125
4	6.72	0.20	0.75	162	06	131
5	7.54	0.08	0.6	178	03	76
6	7.93	0.18	75	142	09	44
7	7.39	0.08	04	142	05	70
8	7.52	0.05	0.7	164	03	39
9	7.77	0.1	0.5	164	04	84
10	7.94	0.11	0.7	160	06	92
11	7.9	0.12	0.4	142	08	62
12	766	0.07	0.4	138	02	84
13	7.73	0.09	0.4	125	10	92
14	7.7	0.08	0.74	185	14	102
15	7.62	0.13	0.74	148	03	130
16	7.52	0.15	0.64	200	06	184

Table:2. *Standard values of soil major elements*

S.No	Elements	High	Medium	Low
1	Nitrogen	>224 kg/acre	112-224 kg /acre	0-112 kg /acre
2	Phosphorous	>24 kg /acre	10-24 kg/acre	0-10 kg acre
3	Potassium	>136	58-136 kg/acre	0-58 kg / acre

5.2. Interpretation of results:

The above results are compared with the standard values and interpreted and determined the nutrient status of each sample shown in table.3

Table.3. The Nutrient status of Soil Samples:

Sample	p^H	E. C	O.C %	N	P	K
1	Neutral	Normal	Low	Medium	Low	Medium
2	Moderately alkaline	Normal	Low	Medium	Medium	Medium
3	Moderately alkaline	Normal	High	Medium	Low	Medium
4	Moderately alkaline	Normal	High	Medium	Low	Medium
5	Moderately alkaline	Normal	High	Medium	Low	Medium
6	Moderately alkaline	Normal	High	Medium	Low	Medium
7	Moderately alkaline	Normal	Low	Medium	Low	Medium
8	Moderately alkaline	Normal	High	Medium	Low	Low
9	Moderately alkaline	Normal	Medium	Medium	Low	Medium
10	Moderately alkaline	Normal	High	Medium	Low	Medium
11	Moderately alkaline	Normal	Low	Medium	Low	Medium
12	Moderately alkaline	Normal	Low	Medium	Low	Medium
13	Moderately alkaline	Normal	Low	Medium	Low	Medium
14	Moderately alkaline	Normal	High	Medium	Medium	Medium
15	Moderately alkaline	Normal	High	Medium	Low	High
16	Moderately alkaline	Normal	Medium	Medium	Low	High

5.3 Determination of Fertility index of the region:

According to nutrient status of the above samples from the Khammam rural region , each macronutrient content was caliculated for its nutrient index by using below formula:

Calculation of the Nutrient Index $= \frac{(N_L \times 1 + N_M \times 2 + N_H \times 3)}{N_t}$

N_L

O. C: - $\frac{6 \text{ X } 1 + 2 \text{ X } 2 + 8 \text{ X } 3}{16} = \frac{6+4+24}{16} = 2.125$

Nitrogen: - $\frac{0+16x2+0}{16} = 2$

Phosphorous: - $\frac{14x1+2x2+0}{16} = 1.125$

Potassium: - $\frac{1x1+13x2+2x3}{16} = 2.6$

Standard Table for Nutrient Index(Reference- *Methods Manuel Department of Agriculture Govt of India***)**

Levels	Reading of Nutrients Index	Interpretation
1	Below 1.67	Low
2	1.67-2.33	Medium
3	Above 2.33	High

From the above analysis the Fertility status of the region

Nutrients	Nutrient Index	Interpretation
O.C	2.12	Medium(1.67-2.33)
N	2.0	Medium
P	1-125	Low(Below 1.67)
K	2.6	High (Above 2.33)

6. Conclusion

From the above observation it is concluded that the representative soil sample are possessing medium fertility status. Especially Organic Carbons and available Nutrients are in medium level and available Potassium is slightly high but available Phosphorous is very low (1.125). The Ph range of the soil are neutral to moderately alkaline the conductivity of all the soil is normal.

By increasing the organic matter by natural means will increase the Organic carbon, Nitrogen & Potassium which in then increase the microbial population. The available Phosphorous depend on phosphate solibulizers present in the soil which have scope for further study to investigate the regions for low available phosphorous and microbial activity in the soil.

Suggestions :

The available Phosphorus is low in the represtataive soil sample and overall fertility index of this region is 1.125 which is low.

Phosphorus is generally present in the combined organic or inorganic salt farm in the soil this farm is unavailable for the plant. If calculate the total phosphate it will be always high but the available phosphate very less this is due to very low microbial conversion or solubulization of phosphate so the level can be increased by increasing microbial activity which involves in solubulization of combined phosphate to available phosphate

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