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LABORATORY MANUAL ON SOIL AND PLANT ANALYSIS

NATIONAL TRAINING ON

Climate Resilient Soil Management Strategies for Sustainable Agriculture

14th October to 3rd November, 2015

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Organized by

Centre of Advanced Faculty Training
Department of Soil Science & Agricultural Chemistry
Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482 004 (M.P.)

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Method of Collection of Soil and Plant Samples for Their Testing

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The information obtained from plant and soil testing which reflect soil and plant health respectively is used for the exploitation of soil and potential for obtaining maximum yield. Assessment of a soils fertility status involves an estimation of its available nutrient status i.e. the portion or amount of nutrient directly available in soil for subsequent uptake by crop plant. The causes of success or failure of this testing are varied usually related to the quality and amount of research data available for calibration, interpretation and recommendations. With reference to this, testing refers to any physical or chemical measurement made on soil/plant. The meaning may be restricted in the sense that it implies rapid chemical analysis used to assess the (available) nutrient status of soil/plant or the meaning may be broader due to use in evaluation interpretation and recommendations. Acceptance of this testing is strongly dependent upon the accuracy with which these tests can be used in solving the localized problems related to crop response and fertilizer use.

Nutrient status of soil and plant acts as a barometer of their health. This can be evaluated in several different ways each having its pros and cons. But the accepted methods include soil testing and plant testing.

Why soil testing ?

- Soils fertility status assessment involves an estimation of its available nutrient status
- It gives the amount of nutrient directly available in soil for subsequent uptake by crop plant.
- Guides to arrive at optimum fertilizer application ratio.
- It is a method of evaluating nutrient status (physico-chemical properties) of the soil i.e. the assessment of the fertility of the soil to determine nutrient deficiencies.
- It is also concerned with environmental quality for the community hazards.

Objectives

(i) To evaluate soil fertility and its productivity by the estimation of level of nutrient (Low, Medium, High).
(ii) Grouping of soil for their classification
(iii) To determine the specific soil problem such as an acidity, alkalinity and sodicity if exist. Subsequently giving recommendation for their correction (Lime/Gypsum requirement etc.)
(iv) To predict the probability of getting maximum response of crops to fertilizers.

Objectives of soil testing:

- To study/ maintain fertility status of a field.
- To predict the probability of obtaining a profitable response of lime & fertilizers.
- To provide basis for recommendation of fertilizers.
- Toevaluatefertilitystatusofsoilofanarea/state/ countryfordevelopedofplansforresearchandeducationwork.
- To study the acidity, alkalinity and salinity problems.
- To determine the suitability of the soil for laying gardens.
- Lime problems.
- Soil survey.

Apparatus and materials:

. *Khurpi* . Spade . Augers
. Plastic bowl . Scale . Rack
. Wooden roller . Mortar-pestle . Sieve
. Polythene/paper/cloth bags . Labels
. Card board cartons . Aluminium boxes

Screw Auger

Tube Auger

To meet objective of soil testing is divided into 4 phases:

1. Collection of soil samples
2. Extraction and determination of the available nutrients
3. Interpretation of analytical results
4. Providing recommendation
5. Follow up of results and evaluation of the recommendations made

Procedure for soil testing

The procedure for testing the soil to meet these objectives are divided into the following phases:

(i) Collection of soil samples and its preparation
(ii) Extraction and determination of nutrients and physico-chemical properties of the soil.
(iii) Interpretation of analytical results
(iv) Recommendation and follow up of results and evaluation of recommendations.

Collection of soil samples

Since soil is a very heterogeneous mass and the greatest source of error is usually soil samples itself hence, the soil sample collected should be representative of the area sampled and should also be uniform. Variations in slope, texture, colour, crops grown, and management levels must be taken in to account. Recently fertilized plot, bunds and channel, spot near tree, wells, compost pits and other non-representative locations must be avoided while sampling. When crops are planted in rows, samples can be drawn in between the lines. The sample should be taken in a zig-zag manner. A representative composite soil samples can be composed of 8 to 20 sub samples from a uniform field (Jones 1988). A common error in soil sampling occurs when the top few cm of soil are dry and are not included in the normal sample. Hence, the value of soil test depends on how well the sample represents a field.

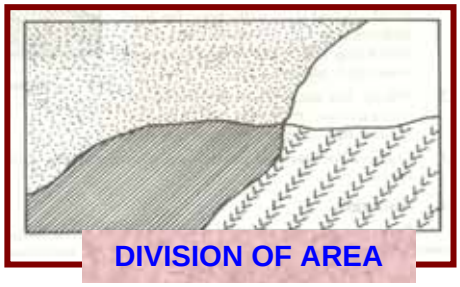
A variety of factors such as depth of sampling, number of cores of composite sample, season, crop etc. influence the collection of soil samples. For field crops a sampling depth upto 15-30 is desired while, 10 cm depth is normally sufficient for sampling for the pasture crops (Rao, 1995). For deep rooted crops, horticultural crops or under dry farming conditions sample from various depths may be preferred. For immobile nutrient, testing of sample to tillage depth may give satisfactory results but for testing of mobile nutrients ($NO+_3^-$, Cl^-, SO_4^{--} etc.) sample should be taken to a depth of 60 cm. In saline alkali soils, salt crust (visible) on the top soil surface should be taken separately along with the depth of sampling recorded. Under intensified cultivation sampling should be done every year at the same time. For sampling of soft and moist soil, the tube auger, spades, or khurpi is quite satisfactory for sampling. A screw type auger is more convenient on hard/dry soil while, post whole auger is useful for sampling excessive wet or rice field. If spade or khurpi is used a V shaped cut may first be made up to the plough layer. A uniform 15 cm thick slice taken out. Auger are generally useful for deep profile sampling.

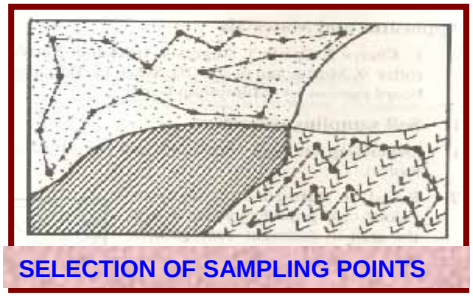
How to take representative soil sample

1. Divide the field into separate units depending on variation in slope, colour, texture, crop growth and management.
2. Remove the debris, rocks, gravels etc from the surface before collecting soil sample.
3. Make a V shape cut into the soil to a depth of sampling (0-15 cm) and obtain 2 to 3 cm thick vertical slices along the depth.
4. Collect 10-15 samples randomly in zig-zag manner from each field.
5. Mix samples by quartering method and approximate 500 g of sample is retained.
6. The sample must be kept in a clear cloth or polythene bag.
7. Label it with suitable description and identification marks.
8. Send the soil samples to soil testing laboratory along with the information sheet.

Information sheet : The soil sample thus collected must be furnished important information like –

1. Sample number
2. Name and address of the farmers.
3. Details of the field and site. Local name field,Khasra no etc.
4. Date of sampling
5. Name of crop and variety to be sown
6. Source of irrigation
7. Whether the crop in the subsequent season will be irrigated or un-irrigated.
8. Name of crops and fertilizer used in previous years.
9. Date of harvest of the previous crop.
10. Any other problem observed in the field.





SELECTION OF SAMPLING POINTS

Preparation of soil sample for testing

1. Spread sample for drying on clean cloth, plastic or brown paper sheet.
2. Remove the stone pieces, roots, leaves & other un-decomposed organic residues from the samples.
3. Large lumps of moist soils should be broken.
4. After air drying the samples should be crushed gently and sieved through a 2 mm sieve.
5. About 250 g of sieved sample should be kept in properly labeled sample bag for testing.

Appropriate time for soil sampling

An ideal time for soil sampling is just after harvest of the rabi crops,

Precautions to be taken during collection of soil sampling

1. Remove all debris from surface before collection of soil sample.
2. Avoid taking sample from upland and low land areas in the same field.
3. Take separate sample from the areas of different appearances.
4. In row crop take sample in between rows.
5. Keep the sample in a clean bag.
6. A sample should not be taken from large area (more than 1-2 ha).
7. Sample for micronutrient analysis must be collected by steel or rust free khurpi/auger and kept in clean polythene bag.

Plant analysis

Plant analysis is used as a diagnostic technique to determine the nutritional status of plants and fertilizer needs. Mineral composition of plant is influenced by many factors which are also to be considered. Fundamental advances in quantitative studies at relationship between nutrient and crop yield have been attempted. In older methods of analysis the quantities of minerals in the manure plant were determined but under the newer concept of analysis only functionally assimilating portion of plant is analyzed. Hence, sampling of the assimilatory portion of the plant is important.

Plant sampling

The particular method to be used will depend upon the kinds of plants to be sampled determinations to be made and over all objectives.

i) Take enough individual plant or plant parts to overcome the factor of plant variability.
ii) If correlation with soils are to be made make sampling distribution representative of a given soil area.
iii) Collected samples should be cleared to avoid contamination from fumes or decomposition.
iv) Suitable methods should be employed for grinding.

If testing is to be done for conforming deficiency/ toxicity as displayed by visual symptoms then 100 to 200 leaves showing particular visual symptoms should be collected.

If the testing is to be used for continued fertilizer guidance then sampling should be done by dividing the whole area into units and then collecting the samples from each unit. Units may be selected on the basis of slope, texture, depth and colour of soil etc. in case of orchards at least 20% of the trees should be sampled. Seven to 10 months old spring cycle leaves be selected. Those leaves should be so selected so that they represent average foliar condition.

In fact plant sampling can be modified depending upon the purpose for which the sample is required. In studying the influence of factors as the stage of growth, cultural practices, manurial treatment or grazing on mineral composition of a crop of pasture whole above ground part of the plant from selected areas will be required. When composition of different species of plants growing on various soil types is needed, the material sample should be restricted to pure species at comparable stage of growth. To study the effect on nutrient uptake the desired investigations of soil types plant should be taken from a small area typical of the soil type and a composite soil sample should also be collected to represent the same area.

Interpretation of the results

Results of foliar diagnosis must be confirmed by plant analysis and concentration of nutrient in the soil. It will give general relationship between growth and quantity of the nutrient absorbed.

Percentage of an element in the plant alone cannot be taken as the basis of judging the deficiency/sufficiency of the element. However, if it is used then physiological age of the leaf must be

taken into consideration. Sometimes the percentage of an element in the plant is to be judged vis-à-vis the percentage of other elements i.e. ratios of the elements are used.

However, the information about the percentage of nutrient in the plant based on considerable amount of experimentation can be used to define the critical level (Cate and Nelson, 1965) i.e. percentage of an element in the plant below which the application of the element in mineral fertilizer form is likely to increase the yield. Moller (1970) have suggested the diagnosis and control of the nutritional disorders increase based on inorganic tissue tests.

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Profile Studies of Deep Black Soils (Vertisols)

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Vertisoils or deep black soils occur globally under various parent materials and environmental conditions (Table 1).

Table 1. Distribution of Vertisols and associated soils

Juris-Diction	Location	Area (m ha)	% of Gross Black soils
Continent	Africa	105.0	38.7
	Asia & far East (Mainly India)	**70.3**	25.9
	Australia	48.8	17.7
	Latin America	27.0	9.9
	North America	10.0	3.7
	Near & Middle East	5.7	2.1
	Europe	5.4	2.0
	TOTAL	**271.4**	**100**
Country	India	**70.3**	25.9
	Australia	48.8	17.7
	Sudan	43.4	16.6
	USA	18.1	6.7
	CHAD	15.5	5.7
	China	11.6	4.3
	Others (in parts)	64.5	23.7
	TOTAL	**271.4**	**100**
India	MS	24.2	34.4
	MP	**21.2**	30.1
	GUJ.	4.9	7.0
	AP	9.4	13.4
	KTK.	5.8	8.2
	TN	2.6	3.7
	RAJ.	1.1	1.6
	UP	1.1	1.6
	TOTAL	**70.3**	**100**
MP	Vertisols	8.0	37.7
	Inceptisols	8.6	40.6
	Entisols	4.2	19.8
	Alfisols	0.4	1.9
	TOTAL	**21.2**	**100**

They clayey soils that shrink and swell extensively upon changing soil moisture conditions. Vertisols exhibit unique morphological properties such as the presence of slickensides, wedge-shaped aggregates, diapir (mukara), and gilgai. Shrink-swell phenomena are the dominant pedogenic processes in Vertisols and are attributed to changes in interparticle and intraparticle porosity with changes in moisture content.

Definition of Vertisols :

Taxonomically for defining Vertisols, there must be

1. A layer 25 cm or more thick with an upper boundary within 100 cm of the mineral soil surface, that has either SLICKENSIDES or WEDGE SHAPED PEDS that have their long axes tilted 10 to 60^{o} from the horizontal; and
2. A weighted average of 30 % or more clay in fine earth fraction either between the mineral soil surface and a depth of 18 cm or in Ap horizon, whichever is thicker and
3. 30 % or more clay in fine earth fraction of all horizons between a depth of 18 cm and either a depth of 50 cm or a densic, lithic or paralithic contact, a duripan, or a petrocalcic horizon if shallower and
4. Cracks that open and close periodically.

Vertisols are significant global resources that serve as the lifeline in subsistence agriculture due to their high productivity.

Efforts towards comprehension and successful utilization are imperative for continued productivity and long term sustainability of these resources for current and future civilizations.

Morphology of a soil is best evaluated from the *in situ* examination of the soil profile. A recently dug pit large enough for observation of a pedon is desirable. Old exposures such as road banks and ditches are acceptable only for preliminary studies because morphological features often become altered after prolong exposure.

Normally the size of profile pit is kept 1.8 m long, 1.2 m wide and 1.8 m deep but for the study of black soils, the width of pit varies from place to place depending on its cyclic wave length of puffs and shelves. It should be kept in mind that at least half wave length covering both, puff and shelve is considered while exposing profile pit in order to study the pattern of cracks and slickensides perfectly.

The profile examination begins with a first approximation and marking of soil horizon boundaries on the profile. Each horizon is then carefully observed and described. Horizon boundaries are relocated as required by the detailed study (Buol *et al*. 1998). Th description sheet containing the columns of site and soil characteristics is filled up by the profile study group during pedon studies.

Vertisols are relatively homogneous in their morphology. Although horizonation is not distinct yet a few horizons above the parent material may be identified as self mulching surface (Ap), blocky subsurface (A12), slickensided horizon and wedge shaped subsoil (Bss).

The depth of these soils may vary from shallow to very deep. Previously the black soils were grouped as shallow (<30 cm) medium (30-100 cm) and deep (>100 cm) but later on Sehgal (2008) modified the depth of shallow soil as less than 50 cm.

Requirement of Vertisols :

Main requirements of Vertisols are the presence of high content of clay (>30 %) and predominance of montmorillonite (2:1 expanding clay). Other important parameters for the development of Vertisols are:

(i) **Parent material** having basalt, argillaceous limestone, marine clays and shale

(ii) **Weathering period** must be extensive for the development of solum with 2:1 expanding clays

(iii) **Weathering environment** should be such that no further weathering of 2:1 expanding clays takes place. Even no inter-layering exists to the extent the properties are destroyed

(iv) **Sequence of events** should continue like churning/mixing, development of argilli-pedoturbation, development of slickensides and formation of wedge shaped structures

Pedogenesis of Vertisols :

1. Separation of blocks : Deep wide cracks separate the soil into strong and massive prism like blocks in the upper part of the pedon that break into angular blocky peds of hard and firm consistence.

(a) ***Cracking of soil*** : During dry season, the soil cracks to the surface due to shrinkage of 2:1 expanding clays that may extend to a depth of 1 metre or more.

(b) ***Falling of surface soil material*** : While cracks are open, surface soil material falls into them by several mechanisms such as animal activity, wind or at the onset of rainy season by water.

2. Hydration of clays : The clay hydrate and due to their high coefficient of expansion and contraction, expand 3 dimensionally on wetting.

(a) ***Expansion of clays*** : Cracks close on the surface but because of the extra material now present in the lower part of the profile, a greater volume is attained and the expanding material presses and slides the aggregates against each other, developing a "lentil" angular blocky structure with slickenside features on the pad surfaces.

(b) ***Shear stress development***: The slipping occurs where shear strength is surpassed by shear stress acting upon a soil mass. The shear stress is a major force caused by swelling and develops when volume expansion results during the wet cycle.

(c) ***Formation of slickensides****:* The slickensides, intersecting or close enough to intersect, also result in wedge shaped structural aggregates, the most characteristics feature of Vertisols which develop with their longitudinal axes inclined at 30 to 60° from horizontal (Sehgal and Bhattacharjee, 1988).

(d) ***Buckeling of land space****:* This expansion buckles the land scape, forming the micro relief called gilgai. The micro basins contain more organic matter than the micro ridges and probably it results from admixtures of subsurface material into micro ridge area and slight erosion of organic rich fines from the ridges to the basins.

3. Incomplete leaching : In most shrink swell soils, the temperature being high, the potential

evapotranspiration suggesting incomplete leaching and inducing the process of calcification in these soils.

Cyclic movement of soil material :

Amongst several processes acting in the formation of Vertisols, the predominant process seems to be haploidization i.e. mixing by argilli pedoturbation. The specific features of such soils are :

1. **Gilgai micro relief** : The term gilgai is an Australian aboriginal term meaning small water hole.

Pedogenic micro topographical features like puffs (microknolls) and shelves (micro basins) develop that remain intimately associated with one another (Bhattacharjee *et al*. 1977), Columbe *et al*. (1996) introduced a term "diapir" meaning a protusion of subjacent soil material which penetrates to the overlying horizons and approaches or reaches the surface. If diapir and gilgai occur, the mound in gilgai is always developed over the diapir.

Hallsworth and Beckman (1969) classified gilgai into 6 types i.e. normal or round, melon hole, Lattice, Linear or wavy, tank or stony but later on Paton (1974) suggested only two types of gilgai i.e. linear and circular (Nuram or Pockmarked) each of which were grouped into 4 types.

α type - Mound and depression equally developed (No shelf present)

β type - Mound of much greater extent than depression (No shelf present)

γ type - Depression of much greater extent than mound (No shelf present)

δ type - Mound, shelf and depression all present

2. **Size of cyclic pedons** : Half cycle linear distance (HCLD) measures the lateral dimension of a cyclic pedon. It may be small, medium or large i.e. below 1, 1 to 2 or above 2 to 3.5 meter, respectively.
3. **Horizon sequence** : In Vertisols, the horizon sequence has been suggested to be A1-Bss-BC-C where "ss" indicates about the presence of slickensides.
4. **Thickness of horizon** : Thickness of A1 in Vertisols varies with the linear frequencies of puffs and shelves of gilgai micro relief.
5. **Horizon boundary (Amplitude)**: It is the difference between vertical distance from the surface of pedon to the lower boundary of crest of cycle and the lowest point of trough of cycle in same pedon. The amplitudes are grouped as low, medium of high according to the vertical distance as below 25, 25 to 75 or above 75 cm, respectively. Shape of apparent topography of the intermittent horizon is also graded as tongued (vertical extent > horizontal distance), wavy (vertical extent approximating the horizontal distance) and smooth (vertical extent < horizontal distances) as suggested by Bartelli (1971).

Age of Vertisols :

It is difficult to assign the Vertisols a place in the genetic scheme of soil classification as there are greater differences of opinion whether they are old, young or remain in equilibrium with the environment.

1. **Views as Vertisols are old** : The end product of a development sequence involves the soils whose B horizon has become so clayey that shrink-swell cycles developed and eventually "swallowed" the A horizon. It is possible because high content of fine clay and high fc/cc ratio may be produced by lessivage on a large scale.
2. **Views as Vertisols are young** : The fate of Vertisol may be to undergo alteration of 2:1 clays to non expanding type of clay. The profile would then cease to churn and eluviation process would dominate. This interpretation suggests that Vertisols are relatively young soils.
3. **View as Vertisols are in equilibrium** : Vertisols remain in equilibrium with their environment and that the 2:1 expanding lattice clays are stable and will persist, barring a climate change. Vertisols then can be considered diagnostic of environments in

which the parent material is basic and gives rise to the formation of 2:1 expanding lattice silicates under the influence of wet dry climate.

Table 2 : Range in characteristics of Vertisols and Vertic Inceptisols

Horizon	Soil colour (10 YR)	Texture	Structure	Special features	Width of cracks (cm)
A. Typic Haplustert (10 YR - 2.5 YR)					
Ap/A11	4/2, 3/3, 3/2, 3/1	C	1f/1m sbk	1c/2c pr-3c pr	2-5
A12	3/3, 3/2, 3/1	C	2m/2c abk	2c pr - 3c pr	2-5
Bss	3/3, 3,2, 3/1, 2/1	C	2m/3c abk	Intersecting lickensides*	1-2
BC	4/4, 3/4	C	2m/2c abk	----do----	0.5-1
C	5/4, 4/4, 4/3	c-gc	2msbk/ massive	-	-
B. Vertic Haplustept (10 YR - 7.5 YR)					
Ap/A	5/2, 4/3, 4/2, 3/2	Cl	gr/1m sbk	1c pr-2c pr	2-2.5
AB	4/3, 3/3	cl-c	1m/2m sbk	----do----	2-2.5
B21	4/3, 3/3, 3/2	cl-c	2m sbk-3m/3c	2c pr - 3c pr or pressure faces/abk slickensides	1.5
B22	6/3, 5/3, 4/4, 4/3	gscl-cl	----do----	-	-
C	7/6, 6/3, 5/3, 4/4	gsl-gscl	1f sbk/ massive	-	-

*or parallelepipeds with long axes tilted from 35° to 55° from horizontal

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Creation of spatial variability maps of soil nutrient using geo-statistical tool

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Soil fertility, a dynamic natural property, fluctuates throughout the growing season of each year due to alteration in the quantity and availability of nutrients by the addition of fertilizers, manure and compost. Due to the bulkiness, weight and lack of technologies to apply manure, it is restricted to fields near the settlements. This always causes over- or under application of the required plant nutrients and therefore, creates a nutrient gradient across the cultivated lands and leads to other undesirable environmental impacts. The resulting spatial variability of soil fertility poses great challenge to land management and reflects in variable yields over farmlands. Some earlier studies showed that the effect of variability of soil properties on crop performance could be detrimental, especially when the fields were patchy. Ideally, application rates should be adjusted based on estimates of the requirements for optimum production at each location because there is high spatial variability of N, P, and K within individual fields. From the literature point of view, it is revealed that high costs spent in classical methods for collecting soil samples and the results have been restricted to only mean values of specific classes. In the old studies latitude and longitude of sampling sites are missing. Keeping this in mind, the present investigation was focused on generation of spatial variability maps of soil nutrients using geo-statistical tool in Arc GIS environment and the use of GPS receivers to map the exact location of sampling sites, which can be used as input data in GIS and run the queries to know various properties of soil.

Methodology

The sampling sites decided randomly distributed over agricultural land of the study area can be perfectly located by considering land use and soil association maps, topography and heterogeneity of the soil type. Field data collection and soil sampling were carried out by using GPS by navigating those points during off season from the agricultural land to avoid the effect of fertilization during crop cultivation. For each main sampling point, 1.0 kg of representative composite soil sample was collected, processed and analyzed for soil nutrients in the laboratory.

Geo-statistical analysis using GIS

In the 1970s a new technique called "Kriging" and its variants were widely recognized as an important spatial interpolation technique in land resource inventories (Hengl *et al.*, 2004). It is a fact that soil properties vary from place to place even within the same field. As a result, the spatial structure can vary at scales that differ by several orders of magnitude from a few meters to hundred kilometers. Such variation with distance can be described well with the help of geo-statistics (Simon *et al.*, 2013). Because "Kriging" assumes the normal distribution for each estimated variable, it is necessary to check whether the nutrient contents in soils are approximately normally distributed or not. A normal distribution was estimated based on skewness values and the variable datasets having a skewness ranged between -1 to 1 were considered normally distributed (Ortiz *et al.*, 2010). The spatial dependency of selected soil parameters was analyzed using semi-variogram analyses with normalized data. Semi-variogram is computed as half the average squared difference between the soil properties of data pairs and semi variance is estimated using equation as:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=a}^{N(h)} [z(x_i) - z(x_i + h)]^2 ,$$

Where *N(h)* is the number of data pairs within a given class of distance and direction, $z(x_i)$ is the value of the variable at the location xi and $z(x_i + h)$ is the value of the variable at a lag of h from the location x_i.

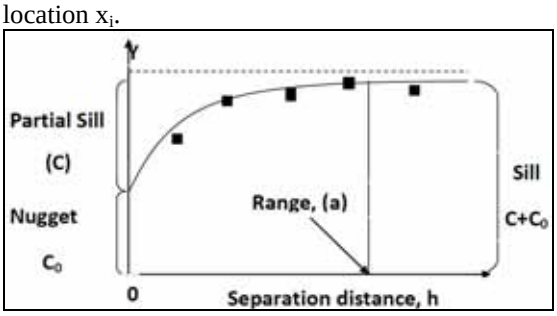
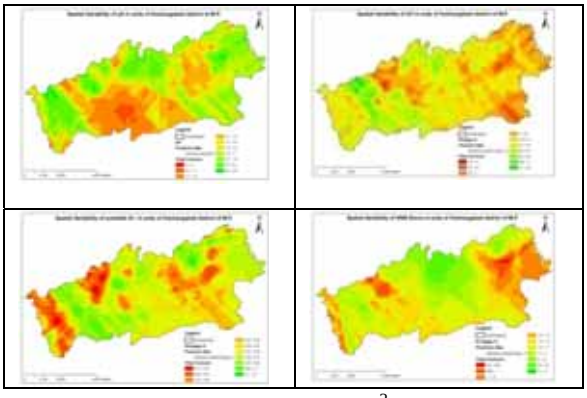


Fig. 1 Semi-variogarm parameters

Then, this was generally fitted with a theoretical model, such as Exponential, Spherical and Gaussian models (Goovarts, 1999). Choice of the best-fitted model was based on the lowest residual sum of square (**RSS)** and the largest



coefficient of determination (R^2). **Nugget** is the variance at distance zero, **sill** is the semi- variance value at which the semi-variogram reaches the upper bound after its initial increase, and range is a value (x axis) at which one variable becomes spatially independent.

The **nugget to sill ratio** was used to define different classes of spatial dependence for the soil properties. According to Cambardella *et al.,* (1994) nugget/sill ratio of 25%, 25-75% and >75% were classified as having strong, moderate and weak spatial dependence, respectively.

Accuracy of the prediction was evaluated through cross-validation approach

Mean absolute error (MAE), and mean squared error (MSE), measure the accuracy of prediction, whereas goodness of prediction (G) measures the effectiveness of prediction criteria (Santra *et al.,* 2008). MAE is a measure of the sum of the residuals (e.g. predicted minus observed) as stated by Voltz and Webster (1990).

$$MAE = \frac{1}{N}\sum_{i=1}^{N}[|z(x_i) - \hat{z}(x_i)|]$$

where z^(xi) is the predicted value at location i. small MAE values indicate few errors. The MAE measure, however, does not reveal the magnitude of error that might occur at any point and hence MSE was calculated,

$$MSE = \frac{1}{N}\sum_{i=1}^{N}[z(x_i) - \hat{z}(x_i)]^2$$

Squaring the difference at any point gives an indication of the magnitude, e.g. small MSE values indicate more accurate estimation, point-by-point. The G measure gives an indication of how effective a prediction might be, relative to that which could have been derived from using the sample mean alone(Agterberg, 1984),

$$G = \left(1 - \sum_{i=1}^{N}\frac{[z(x_i) - \hat{z}(x_i)]^2}{\sum_{i=1}^{N}[z(x_i) - \bar{z}]^2}\right) \times 100$$

where z is the sample mean. If G = 100, it indicates perfect prediction, while negative values indicate that the predictions are less reliable than using sample mean as the predictors (Schloeder *et al.*, 2001).

Case study

From Hoshangabad district, 305 soil samples were collected using GPS and analysed for soil properties. Then spatial variability maps of pH, OC Zn and B using geo-statistical tool in GIS environment was generated.

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Determination of pH and Electrical Conductivity in Soil Samples

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Determination of pH is actually a measurement of hydrogen ions activity in soil – water system. It is defined as negative logarithm of the hydrogen ion activity. Mathematically, it is expressed as:

$$pH = - \log a H^+$$

The pH value of a soil is an indication 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 which also serves as salt bridge. Now a day, most of the pH meters have single combined electrode. 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 tablet in 100 ml distilled water, It is necessary to prepare fresh buffer solution after few days. In absence of buffer tablet, a 0.05 M potassium hydrogen phthalate solution can be used which gives a pH of 4.0 (Dissolve 10.21 g. of A.R. grade potassium hydrogen phthalate in distilled water and dilute to 1 litre. Add 1 ml of chloroform or a crystal of thymol per litre as a preservative).

Procedure :

(a) Soil to water ratio of 1:2 (pH_2)

Take 20 g soil in 100 ml beaker and add 40 ml. of distilled water to it. The suspension is stirred at a regular interval for 30 minutes. Determine the pH by immersing electrodes in suspension. For soils containing high salts, the pH should be determined by using 0.01M calcium chloride solution. (Dissolve 0.110 g of $CaCl_2$ in water and dilute to 1 litre).

(b) Saturates soil paste (pHs)

Add small amount of distilled water to 250g of air dried soil. Stir the mixture with a spatula. At saturation, the soil paste glistens and flows slightly when the container is tapped it slides freely and ensures cleanly off the spatula. After mixing, allow the sample to stand for an hour. If the paste has stiffened markedly or lost its glistening, add more water or if free water has collected on the surface of the paste, add an additional weighed quantity of dry soil and mix it again. Then insert the electrode carefully in the paste and measure the pH.

(c) Saturation extract (pHe)

The soil is extracted using vacuum extractor and the pH is measured in the saturation extract.

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

Electrical Conductivity :

Amount of soluble salts in a sample is expressed in terms of the electrical conductivity (EC) and measured by a conductivity meter. The instrument consists of an AC solubridge or electrical resistance bridge and conductivity cell having electrodes coated with platinum black. The Instrument is also available as an already calibrated assembly (Solubridge) for representing the conductivity of solutions in dSm^{-1} (deci Siemen per meter) at 25^0C.

Principle

A simple wheatstone bride 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.

$$\frac{r_1}{r_2} = \frac{r_3}{r_4} \quad \text{or} = r_3 \quad \frac{r_1}{r_2} \quad X\ r_4$$

Since conductivity is reciprocal of receptivity, it is measured with the help of r_3.

Reagents :

Potassium chloride: Dissolve 0.7456g dry potassium chloride (AR) in distilled water and make up the volume to one litre.

Procedure :

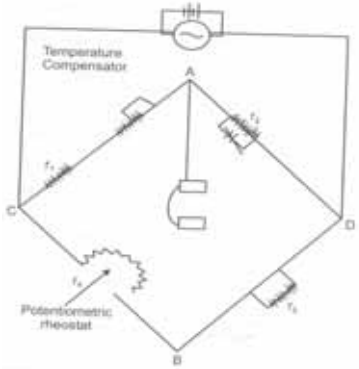
Take 20 g of soil in 100 ml beaker, add 40 ml of distilled water and shake intermittently for 30 minutes. Determine the conductivity of the supernatant liquid with the help of conductivity meter. The electrical conductivity of saturation extract (E.C.) is also determined for salinity ratings.

EC Effect (dS m^{-1})

<1 - No deleterious effect on crop
1-2 - Critical for salt sensitive crops
2-3 - Critical for salt tolerant crops
>3 - Injurious to most crops

pH Meter :-

Electrical Conductivity Meter :-



Determination of Organic Carbon Content in Soil

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The majority of mineral surface soils range from 1.2 to 3.5% organic carbon. Since soil organic matter averages about 58% carbon, it follows that soils generally range from about 2 to 6 % organic matter (% O.M. = %C x 1,724. The factor 1.724 = 100/58). There is also a close relationship between carbon and nitrogen in soils. Most organic matter average about 5% nitrogen so that the N : C ratio is 1:11.6. Therefore by multiplying the soil organic matter percentage by 0.05 an approximate value for the soil nitrogen, percentage is obtained.

In soil the chief source of some of the nutrients essential for plant growth is organic matter, such nutrients are N, S and boron is also largely derived from organic matter.

Principle :

A suitable quantity of the soil is digested with chromic acid and sulphuric acid making the use of heat of dilution of sulphuric acid soil is digested and organic matter of the soil is oxidized. Excess of chromic acid left over unreduced by the organic matter of the soil is determined by a titration with standard Ferrous Ammonium sulphate solution using diphenylamine as indicator.

In this exercise, chromic acid in the presence of excess H_2SO_4 is to be used as an oxidizing agent for oxidizable organic matter of the soil. The heat of dilution of H_2SO_4 works as a standardized ferrous sulphate solution.

$$4\,Cr^{6+} + 3C \longrightarrow 4Cr^{+++} + 3C^{4+}$$

$$2H_2Cr_2O_7 + 3C + 6H_2SO_4 \longrightarrow 2Cr_2(SO_4)_3 + 3CO_2 + 8H_2O$$

$$K_2Cr_2O_7 + 4\,H_2SO_4 \longrightarrow K_2SO_4 + 2Cr_2(SO_4)_3 + H_2O + 3O$$

$$2FeSO_4 + H_2SO_4 + O \longrightarrow Fe_2(SO_4)_3 + H_2O$$

Apparatus and Reagents :

1. 500 ml conical flasks.
2. Pipette
3. Burette
4. Phosphoric acid 85%.
5. Sodium fluoride 2%.
6. Sulphuric acid 96 % containing 1.25 % Ag_2SO_4.
7. Standard 1N $K_2Cr_2O_7$ – 49.04 g/liter.
8. Standard 0.5 N Fe $(NH_4)_2$ $(SO_4)_2$. $6H_2O$ 196 g in 800 ml water containing 20 cc H_2SO_4 and diluted to1 litre.
9. Diphenylamine – 0.5g in 20cc water and add 100 ml conc. H_2SO_4.

Procedure :

1. Weigh 1g soil sample in 500 ml conical flask. Add 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml conc. H_2SO_4 (containing Ag_2SO_4). Mix thoroughly and allow reaction to proceed for 30 minutes.
2. Dilute the reaction mixture with 200 ml water and 10 H_3PO_4 add 10 ml of NaF solution and 2 ml of diphenylamine.
3. Titrate the solution with standard FAS to a brilliant green colour. A blank without soil should be run simultaneously.

Observations & Results :

Weight of sample	- 1 g
Normality of $K_2Cr_2O_7$ used	- 1 N
Vol. of $K_2Cr_2O_7$	- 10 ml
Normality of FAS	- 0.5 N

$$OC\ (\%) = \frac{10}{Blank}\ (Blank - Reading)\ X\ \frac{0.003 \times 100}{Wt.\ of\ soil}$$

Limits :

Low	:	< 5.0 g OC kg^{-1}
Medium	:	5.0 to 7.5 g OC kg^{-1}
High	:	> 7.5 g OC kg^{-1}

Determination of Available Nitrogen in Soil

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Available Nitrogen in Soil (Alkaline Permanganate Method) :

Principle :

A known weight of the soil is mixed with alkaline potassium permanganate ($KMnO_4$) solution and distilled. The organic matter present in soil is oxidized by the nascent oxygen, liberated by potassium permanganate, in the presence of sodium hydroxide and the released ammonia is condensed and absorbed in known volume of a boric acid with mix indicator to form ammonium borate, the excess of which is titrated with a standard sulphuric acid.

Reactions involved:

I. Distillation :

$$2\,KMnO_4 + H_2O \xrightarrow[\text{medium}]{\text{Alkaline}} 2\,MnO_4 + 2KOH + 3O^-$$

(Nascent oxygen)

Oxidation

$$R.CHNH_2COOH + O^- \longrightarrow R.CO.COOH + NH_3$$

Organic-N fraction **(**Ammonia)

Distillation

$$NH_3 + H_2O \longrightarrow NH_4OH$$

Absorption

$$3NH_4OH + H_3BO_3 \longrightarrow (NH_4)_3BO_3 + 3H_2O$$

(Green colour)

II. Titration

$$2(NH_4)_3BO_3 + 3H_2SO_4 \longrightarrow 2(NH_4)_3SO_4 + 2\,H_3BO_3$$

(Pink colour and original)

Equipment and apparatus :

1. KEL PLUS Automatic Nitrogen Estimation System

The said instrument is used for determination of available nitrogen in soil. It consists of the following:

- **Automatic Distillation System (Model Classic DX):** It is fully automatic distillation system with programmable auto run digital features, with automatic dilution and addition of boric acid, NaOH and $KMnO_4$. Both modes (auto and manual) are available for distillation reagents addition.
- **Refrigerated Water Cooling System for Condenser (Model Kel Freeze):** It is refrigerated water cooling system for distillation and condensing system with inbuilt compressor and re-circulate pump.

2. Electronic balance
3. Burette
4. Conical flask
5. Distilled water

Reagents :

1. 0.32 % potassium permanganate ($KMnO_4$) solution.
2. 2.5 % sodium hydroxide (NaOH).
3. 2 % boric acid solution containing 20 - 25 ml of mixed indicator / liter.
4. Mixed indicator: 0.066g methyl red + 0.099g bromocresol green dissolve in 100 ml of 95 % alcohol.
5. 0.02 N sulphuric acid (H_2SO_4).

Procedure :

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

Calculations :

$$\textbf{Available N (kg ha}^{-1}\textbf{)} = \frac{\text{R (Titer reading - Blank reading)} \times \text{Normality of acid} \times \text{Atomic weight of nitrogen} \times \text{Weight of one hectare of soil}}{\text{Sample weight (g)} \times 1000}$$

$$= \frac{R \times 0.02 \times 14 \times 2.24 \times 10^6}{5 \times 1000}$$

Factor = R x 125.44

Interpretation of results :

Available N (kg ha^{-1})		**Soil rating**
< 280	:	Low
280-560	:	Medium
> 560	:	High

Determination of Total Nitrogen in Soil and Plant Samples

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Total nitrogen is estimated by the micro-Kjeldahl method as per procedure suggested by AOAC (1995).

Preparation of plant and soil samples :

The plant analysis has been considered as a superior diagnostic technique for mineral content. Whole plant is dried in open air for few days after that it was further dried in hot air oven at about 60 ± 2° C for eight to ten hours per day to achieve complete drying. After drying, whole plant is powdered with the help of a grinder, passed through 2 mm stainless steel sieve and used for chemical assay. The soil sample from definite depth was randomly collected from the field with the help of screw auger. All the possible technical precautions as prescribed for standard soil sampling were also taken. Samples were brought to the laboratory, air-dried in the shade and grounded by wooden roller, thereafter sieved through 2 mm stainless steel sieve and stored in polythene bags and used for chemical assay.

Principle :

Nitrogen in samples like plant and soil exists in a very complicated bonding structure. During digestion, a known weight of the plant/soil samples in the presence of sulphuric acid with catalyst mixture under high temperature is digested where complicated structures are broken to simple structure, thereby releasing nitrogen in the form of ammonium radical (NH_4^+). During distillation in presence of sodium hydroxide, the released ammonia is condensed and absorbed in known volume of a boric acid with mix indicator to form ammonium borate, the excess of which is titrated with a standard sulphuric acid.

The micro-Kjeldahl method consists of the three steps;

1. Digestion
2. Distillation and
3. Titration.

Equipment and apparatus :

1. KEL PLUS Automatic Nitrogen Estimation System :

The said instrument is used for determination of nitrogen. It consists of the following :

- **Macro Block Digestion System (Model KES 12L):** This digestion system is suitable for soil, plant, water, pesticides, fertilizers, food and feed samples. It is microprocessor based automatic twelve place macro block digestion system with temperature controller fitted with sensor break protection (Microprocessor based) feature and temperature range from 50-450 ^{0}C.
- **Acid Neutralizer Scrubber (Model KEL VAC):** It is used to neutralize the acid fumes, which are absorbed in 15% sodium hydroxide and dissolved in water stored in the system tank. After every 2 cycles of digestion, the 15% sodium hydroxide solution is replaced and after 3 cycles of digestion, acid fumes dissolved in water tank is drained off and refilled with fresh water in the system tank.
- **Automatic Distillation System (Model Classic DX):** It is fully automatic distillation system with programmable auto run digital features, with automatic dilution and addition of boric acid and NaOH. Both modes (auto and manual) are available for distillation reagents addition.
- **Refrigerated Water Cooling System for Condenser (Model Kel Freeze):** It is refrigerated water cooling system for distillation and condensing system with inbuilt compressor and re-circulator pump.

1. Electronic balance
2. Burette
3. Pipette
4. Conical flask
5. Measuring cylinder
6. Distilled water

Reagents :

1. Concentrated sulphuric acid (H_2SO_4).
2. Catalyst mixture: Mix with 250 g potassium sulphate (K_2SO_4), 50 g cupric sulphate ($CuSO_4$. 5 H_2O) and 5 g metallic selenium powder in the ratio of 50:10:1.
3. 40 % sodium hydroxide (NaOH).
4. 4 % boric acid containing 20 - 25 ml mixed indicator /liter.
5. Mixed indicator: 0.066 g methyl red + 0.099 g bromocresol green dissolve in 100 ml of 95 % alcohol.
6. 0.02N sulphuric acid (H_2SO_4).

Procedure :

I. Digestion :

- Weigh 0.5 g of prepared plant sample or 1 g of soil sample and transfer it to the digestion tube.

- Add 10 ml of concentrated sulphuric acid and 5 g of catalyst mixture to the sample.
- Load the digestion tubes in to the digester and heat the digestion block.
- Switch on the digestion unit and set the initial temperature 100 ^{0}C till frothing is over.
- Then block temperature is raised to 400 ^{0}C. The effective digestion starts only at 360 ^{0}C and beyond 410 ^{0}C.
- The sample turns light green colour or colorless at the end of the digestion process.

II. Distillation :

- After cooling the digestion tube, load the tube in distillation unit and other side of hose keep 20 ml of 4 % boric acid with mixed indicator in 250 ml conical flask.
- 40 ml NaOH (40 %) is automatically added by distillation unit programme.
- The digested sample is heated by passing steam at a steady rate and the liberated ammonia absorbed in 20 ml of 4 % boric acid containing mixed indicator solution kept in a 250 ml conical flask.
- With the absorption of ammonia, the pinkish colour turns to green.
- Nearly 150 ml of distillate is collected in about 8 minutes.
- Simultaneously, blank sample (without plant/soil) is to be run.

III. Titration :

- The green colour distillate is titrating with 0.02N sulphuric acid and the colour changes to original shade (pinkish color).
- Note the blank & sample titer reading (ml) and calculate the total nitrogen content present in plant/soil samples.

Crude protein content :

The total nitrogen is estimated by micro-Kjeldahl method as per procedure suggested by AOAC (1995) and the crude protein is calculated by the following formula:
Crude protein content (%) = micro-Kjeldahl nitrogen content (%) x 6.25 (based on the assumptions that nitrogen constitutes 16 % of protein).

References :

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Calculations :

$$\textbf{Nitrogen content in plant (\%)} = \frac{\textbf{R}\text{ (sample titer-blank titer) x Normality of acid x Atomic weight of nitrogen x 100}}{\text{Sample weight (g) x 1000}}$$

$$= \frac{\text{R x 0.1 x 14 x 100}}{\text{0.5 x 1000}}$$

$$\textbf{Factor} = \text{R x 0.28}$$

$$\textbf{Nitrogen content in soil (\%)} = \frac{\textbf{R}\text{ (sample titer-blank titer) x Normality of acid x Atomic weight of nitrogen x 100}}{\text{Sample weight (g) x 1000}}$$

$$= \frac{\text{R x 0.1 x 14 x 100}}{\text{1 x 1000}}$$

$$\textbf{Factor} = \text{R x 0.14}$$

Determination of Phosphorous in Soil and Plant Samples

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The phosphorus is an essential plant nutrient and it occurs in many different forms. Therefore, a reliable procedure for measuring the amount both in soil as well as in plant is needed. There are many methods available for the determination, however, colorimetric measurement is presented here:

Principle :

Phosphorus is extracted from the soil with 0.5 m $NaHCO_3$ at a nearly constant pH of 8.5. The phosphate ion in solution treated with ascorbic acid in an acidic medium provides a blue colour complex. Measurement of the quantitative determination of phosphorous in soil (Olsen's *et al*., 1954)

Reagents :

1. **0.5 M Sodium bicarbonate ($NaHCO_3$) solution:** Dissolve 42 g of $NaHCO_3$ in distilled water to get one litre solution and adjust the pH of the solution to 8.5 by small quantity of NaOH.
2. **Activated Charcoal:** Darco G-60 (P- Free)
3. **5 N Sulphuric acid (H_2SO_4) Solution:** Add 141 ml of con. H_2SO_4 to 800 ml of distilled water. Cool the solution and dilute to one litre with distilled water.
4. **Reagent A:**
   - Dissolve 12.00 g of ammonium paramolybdate in 250 ml of distilled water.
   - Dissolve 0.2908 g of potassium antimony tartrate ($KSbO.C_4H_4O_6$) in 100 ml distilled water.
   - Above both solution mix thoroughly and made one litre in volumetric flask with the help of distilled water.
   - Add these dissolved reagents to one litre of 5N H_2SO_4.
5. **Ascorbic acid working solution (Reagent B):** Dissolve 1.056 g of ascorbic acid in 200 ml of **reagent A** and mix. This ascorbic acid (**reagent B**) should be prepared as required because it does not keep more than 24 hours.
6. **Standard phosphate solution**: Weigh 0.4393 g of potassium dihydrogen phosphate (KH_2PO_4) into one litre volumetric flask. Add 500 ml of distilled water and shake the contents until the salt dissolves. Dilute the solution to one litre with distilled water to get 100 ppm P solution. Dilute 20 ml of 100 ppm P solution to one litre to get form-working solution of 2 ppm.

Preparation of standard curve :

- Take different concentration of P (0, 1, 2, 3, 4, 5, etc ml of 2 ppm standard P Solution) in 25 ml volumetric flasks.
- Add 5 ml of the 0.5M $NaHCO_3$ extracting solution to each flask, and acidify with 5N H_2SO_4 drop by drop.
- Add about 10 ml distilled water and 4 ml of reagent 'B', then shake the solution.
- Make the volume 25 ml by distilled water.
- The intensity of blue colour is read on spectrophotometer at 660 nm wavelengths after 10 minutes.
- Plot the curve by taking P concentration on X axis and colorimeter reading on Y axis. Repeat the process till you get straight line relationship.
- Calculate the factor i.e. 1 colorimeter reading is equal to how much ppm of phosphorus?

Procedure :

- Take 2.5 g of soil sample in 150 ml conical flask and 0.5 g Darco G-60 activated charcoal.
- Then add 50 ml of 0.5 M $NaHCO_3$ solution and shake the solution for 30 minute in a shaker. Similar processes run for a blank without soil.
- Filter the suspension through the Whatman no. 40 paper.
- Take 5 ml aliquot of the extract in a 25 ml volumetric flask, and acidify with 5N H_2SO_4.
- Add small quantity of distilled water, and then add 4 ml of reagent B.

- The intensity of blue colour is read on spectrophotometer at 660 nm wavelengths after 10 minutes.

Observations :

1. Weight of soil sample : 2.5 g
2. Volume of extractant used : 50 ml
3. Volume of filtrate used : 5 ml
4. Absorbency : R
5. Absorbency from standard curve : A
6. Concentration of P for absorbency A : B ppm

Calculation :

$$\text{Available P (kg ha}^{-1}\text{)} = \frac{\text{R x F x 50 x 2.24}}{\text{5 x 2.5}}$$

Where, F (factor) = B / A

Limits of available P in soil :

Very low	:	Less than 5 P kg ha^{-1}
Low	:	5-10 P kg ha^{-1}
Medium	:	10-20 P kg ha^{-1}
High	:	20-40 P kg ha^{-1}
Very high	:	More than 40 P kg ha^{-1}

Determination of total phosphorus in plant :

Principle : Vanadate molybdate and orthophosphates react to give a yellow colour complex in acidic medium. The intensity of colour provides the basis of quantitative measurement of total P in plant (Koenig and Johnson, 1942).

Apparatus and reagents :

- Colourimeter/spectrophotometer
- 50 ml volumetric flask
- ammonium molybdate ammonium vanadate (in NHO_3) solution : Dissolve 2.5 g $(NH_4)_6 Mo_7O_2.4\ 4H_2O$ in 400 ml distilled water. Dissolve 1.25 g of ammonium vanadate in 300 ml boiling water. Add the ammonium vanadate solution to the ammonium molybdate solution and cool to room temperature. Add 250 ml conc. NHO_3 and dilute to 1 lit.
- Phosphate standard solution : Dissolve 0.2195 g KH_2PO_4 and dilute to 12%. This solution contains 50µg P/ml.

Procedure :

Preparation of standard curve :

- Transfer 0, 1, 2, 3, 4 and 5 ml of 50 ppm P solution to 50 ml volumetric flasks in order to get 0, 50, 100, 150, 200 and 250 µg P.
- Add 10 ml vanadomolybdate reagent make up the volume and mix the content thoroughly.
- Read the transmittance/absorbance at 420 mµ (blue filter).
- Plot the reading against µg P and calculate the factor (F).

Digestion of plant material :

Take one gram of plant material in digestion flask. Add 10-15 ml of Diacid (3:1: Nitric acid : Perchloric acid) mixture and swirl the content in 150 ml volumetric flask. Place the content on hot plate till the digestion is over. Filter the solution in 100 ml conical flask, wash the residue on filter paper several times with the hot water. Make up the volume with distilled water, store the solution in air tight container.

Estimation :

- Transfer 10 ml dilute in 50 ml volumetric flask.
- Add 10 ml ammonium molybdate vanadate solution shake the content.
- Make up the volume and record the reading as per the procedure under preparation of standard curve.

Calculation :

50 µg = R
1 R = 50/R µg (Factor)

$$\text{Total (\%) P} = \frac{\text{Factor (F) x Reading x 100x100 sample}}{\text{10000 x 1000 x 10 x 1}}$$

Reference :

- Koenig, R.A. and Johnson, C.R. (1942). Colorimetric determination of biological materials Ind. Eng. Chem. Analyt. Edn. 14 : 155-156.
- Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. U.S. Dept. Agric. 939 : 1-19.

Spectrophotometer

Determination of Potassium in Soil and Plant Samples

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The available potassium i.e. 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.

1. 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 lower energy level, light at characteristic wave length is emitted. Excited atoms or ions give line radiation at very definite wave length and thus K gives at 404.4 and 767 mμ. The flame photometer employees a relatively low temperature excitation and measures with a photocell the emission intensity which is proportional and to concentration in selected wave length (767 mμ) and for this red filter is used.

2. Apparatus and reagents:

a) Flame photometer with red filter,
b) Pipette, volumetric flasks and conical flask (100 ml).

3. Reagents :

(a) Neutral Normal Ammonium Acetate :

Add 58 ml of glacial acetic acid to about 600 ml H2O and then add 70 ml of concentrated ammonia (sp. gr 0.90) Dilute the solution to one litre. Then adjust pH of solution at 7.0 with the help of ammonia or acetic Acid or this can be prepared by dissolving ammo. Acetate (CH_3COONH_4) (77.08 eq.wt.) directly in H_2O and volume to be made one litre and then adjust the pH 7.0 .

(b) Standard Potassium Solution :

Dissolve 1.9066 g of dried KCl (AR) in distilled water and dilute to one litre. This is 1000 mg kg-1 K solution. 100 ml of this solution diluted to 1 lit. to make 100 ppm K solution.

4. Preparation of the standard curve :

Take 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml of 100 mg kg-1 K solution in different 25 ml volumetric flasks. Make up the volume with 1N NH_4OAc Soln. Adjust the flame photometer reading at zero with blank (zero K) solution and at 100 for 40 mg kg-1 K solution. Take the flame photometer readings for every dilution. Plot the standard curve on graph paper by taking K concentration on X axis and flame photometer reading an y axis. This will give a factor (F) of 1 flame photometer reading = 0.4 mg kg-1 K.

5. Procedure :

Take 5g soil in 100 ml conical flask and add 25 ml of 1N NH_4OAc Soln. Shake the content for 5 minutes and then filter through Whatman No.1 filter paper. Potassium extract is measured by flame photometer after calibration.

6. Calculation :

$$\text{Available K (kg ha}^{-1}) = \frac{\text{RxFx25x100x20x1.121}}{\text{5 x 1000}}$$

$$= \text{R x F x 11.217.}$$

Limits of available K in soil :

Very low	:	Less than 200 K kg ha^{-1}
Low	:	200 – 250 K kg ha^{-1}
Medium	:	250 – 400 K kg ha^{-1}
High	:	400 – 600 K kg ha^{-1}
Very high	:	More than 600 k kg ha^{-1}

8. Precaution :

a) These should not be any turbidity or suspended particles in extract, it will chock the capillary feeding tube .
b) The gas and air pressure should be constant.
c) If sample reading goes beyond 100 then dilute the extract.

9. Determination of k in plant sample :

(a) Wet digestion :

Place 1-2g of ground plant sample in 100ml digestion flask. Add 20-25 ml of acid mixture Acid mixture 750 ml conc. HNO_3 + 150 ml conc H_2SO_4 + 300 ml of $HClO_4$ and mix the contents of the flask by swirling well. Heat the flask at a low temp and then slowly increase the flame or temp. of hot plate. Completion of digestion is confirmed when liquid is colorless. After cooling, add 20-25 ml H_2O and filter

through whatman No.40 into a 100 ml/250 ml volume flask and make up the volume.

(b) Determination of K :

Take the aliquot and get the reading of K through flame photometer using red filter and calculate the amount of K in the plant sample on the oven dry matter basis.

K (%) in plant sample = $X \times 4 \times 10^{-3}$

References :

Black, C.A. (1965) Methods of soil analysis Part I Am. Soc. Agron. Inc. Publi. Madison Wisconsin USA.

Flame Photometer :-

Determination of Sulphur Content in Soil and Plant

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Principle:

Besides some amount in the soil solution, available sulphur in mineral soils occurs mainly as adsorbed $SO_4^=$ ions. Phosphate ions (as monocalcium phosphate) are generally preferred for replacement of the adsorbed $SO_4^=$ ions. The extraction is also carried out using $CaCl_2$ solution. However, the former is considered to be better for more efficient replacement of $SO_4^=$ ions. Use of Ca salts have a distinct advantage over and leads to easy filtration $SO_4^=$ in the extract can be estimated turbid metrically using a colorimeter/spectrophotometer.

A major problem arises when the amount of extracted sulphur is too low to be measured precisely.; To overcome this problem, addition of seed solution of known S concentration is added to the extract to raise concentration to easily detectable level. Sulphur in the extract can also be estimated by a colorimetric method using barium chromate (Nemeth 1964; Palaskar et al. 1981), but the turbidimetric method (Chesnin and Yien 1950) given below is mainly used in the soil testing laboratories.

Instruments :

(i) Colorimeter or spectrophotometer or autoanalyzer.
(ii) Mechanical shaker

Reagents

1. **Mono-calcium phosphate extracting solution (500 mg P L^{-1}):** Dissolve 2.035 g of $Ca(H_2PO_4)_2.H_2O$ per litre.
2. **Gum acacia-acetic acid solution:** Dissolve 5 g of chemically pure gum acacia powder in 500 mL of hot water and filtered in hot condition through Whatman No. 42 filter paper. Cool and dilute to one litre with dilute acetic acid.
3. **Barium chloride:** Pass AR grade $BaCl_2$ salt through 1 mm sieve and store for use.
4. **Standard stock solution (2000 mg S L^{-1}):** Dissolve 1.089 g of oven dried AR grade potassium sulphate per 100 mL.
5. **Working standard solution (10 mg S L^{-1})**: Measure exactly 2.5 mL of the stock solution and dilute to 500 mL.
6. **Barium sulphate seed suspension:** Dissolve18 g of AR grade $BaCl_2$ in 44 mL of hot water and add 0.5 mL of the standard stock solution (given above). Heat the contents to boiling and then cool quickly. Add 4 mL of gum acacia-acetic acid solution to it. Prepare a fresh seed suspension for each estimation every day.
7. **Dilute nitric acid (approx 25%):** Dilute 250 mL of AR grade conc. HNO_3 to one litre.
8. **Acetic-phosphoric acid:** Mix 900 mL of AR grade glacial acetic acid with 300 mL of H_3PO_4 (AR grade).

Procedure :

1. Weight 20 g of soil sample in a 250 mL conical flask.
2. Add 100 mL of the monocalcium phosphate extracting solution (500 mg P L^{-1}) and shake for one hour. Filter through Whatman No. 42 filter Paper.
3. Measure 10 mL of the clear filtrate into a 25 mL volumetric flask.
4. Add 2.5 mL of 25% HNO_3 and 2 mL of acetic-phosphoric acid. Dilute to about 22 mL, stopper the flask and shake well.
5. Shake the $BaSO_4$ seed suspension and then add 0.5 mL of it and 0.2 g of $BaCl_2$ crystals. Stopper the flask and invert three times and keep.
6. After 10 minutes, invert 10 times and keep. After another 5 minutes, invert 5 times.
7. Allow to stand for 15 minutes and then add 1 mL of gum acacia-acetic acid solution.
8. Make up the volume, invert three times and keep aside for 90 minutes.

9. Invert 10 times and measure the colour intensity at 440 nm (blue filter).
10. Run a blank side by side.

Preparation of standard curve for S :

1. Place 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 ml portions of the working standard solution (10 mg S L^{-1}) into a series of 25 ml volumetric flasks to obtain 25, 50, 75, 100, 125 and 150 µg S.
2. Proceed to develop turbidity as described above for sample aliquots.
3. Read the colour intensity and prepare the curve by plotting readings against sulphur concentration (In µg in the final volume of 25 ml).

Calculation :

$$\text{Available S in soil (mg kg}^{-1}) = \frac{R \times 100}{10 \times 20}$$

Where, r stands for the quantity of S in mg as obtained on X-axis against a reading.

Determination of total sulphur in plant:

Sulphur is an essential plant nutrient and occurs in many different forms. The procedure for total sulphur estimation is as follows :

Digestion of plant material :

Take one gram of plant material in digestion flask. Add 10-15 ml of Diacid (3:1: Nitric acid : Perchloric acid) mixture and swirl the content in 150 ml volumetric flask. Place the content on hot plate till the digestion is over. Filter the solution in 100 ml conical flask, wash the residue on filter paper several times with the hot water. Make up the volume with distilled water, store the solution in air tight container.

Estimation :

Take 10 ml aliquot from extract and proceed as per the method described under preparation of standard curve (Bardsley and Lancaster, 1960).

Calculation :

5 µg = R
1 R = R/5 µg (Factor)

$$\text{Total S (\%)} = \frac{\text{Factor x Sample R x1000 x 100 x 100}}{\text{1000 x 10 x 1}}$$

References :

- Arora, C.L. and Bajwa, M.S. (1994). *Curr. Sci*. 66 : 314-316.
- Bardsley, C.S. and Lancaster, J.P. (1960). Proc. Soil Sci. Soc. Am. 24 : 265.
- Chesnin, L. and Yien, C.N. (1951). Proc. Soil Sci. Soc. Am. 15 : 149.

Determination of Micronutrients in Soil and Plant Samples by Atomic Absorption Spectrophotometer

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All atoms can absorb light at certain discrete wavelengths corresponding to the energy requirement of the particular atom. When at ground state the atom absorbs light it is transformed into the excited state. It is the same atom containing more energy. This energy is measured in relation to the ground state and a particular excited state say for example in case of Na may be 2.2 eV (electron volts) above the ground state.

Each transition between different electronic energy states is characterized by a different energy and by a different wavelength. These wavelengths are sharply defined and when a range of wavelengths is surveyed, each wavelength shows as a sharp energy maximum (a spectronic line). These characteristic lines distinguish atomic spectra. The lines, which originate in the ground state of atom, are most often of interest in atomic absorption spectroscopy. These are called the resonance lines. The atomic spectrum, characteristic of each element, then comprises a number of discrete lines, some of which are resonance lines. Most of the other lines arise from excited states rather than the ground state. The lines of excited states are not useful generally in atomic absorption analysis as most of the atoms in a practical atomizer are found in the ground state.

The relationship of light absorbed by the atom in ground state and their concentration in the solution is defined in the fundamental laws of light absorptions.

Lambert's Law : The portion of light absorption by a transparent medium is independent of the intensity of the incidence light and each successive unit thickness of the medium absorbs an equal fraction of the light passing through it.

Beer's Law : Light absorption is proportional to the number of absorbing atoms in the sample.

The combined Beer - Lambert law may be given as :

$$I_t = I_o - (abc)$$

$$\text{thus, } \log_{10} \frac{I_o}{I_t} = abc = \text{absorbance}$$

Where, I_o = incident radiation power
I_t = transmitted radiation power
a = absorption coefficient
b = length of absorption path
c = concentration of absorbing atoms

i.e. the absorbance is proportional to the concentration of the elements for a given absorption path length at any given wave length.

In principle, it might be possible to calculate the concentration directly from the above equation. In practice, however, the a and b are constants hence the variation of results is directly related the concentration of atoms. For analysis, the absorbance of different concentration of standard solution is first measured with the help of atomic absorption spectrophotometer and then the results of unknown samples are compared with the standards and thus concentration of unknown sample is calculated.

Atomic absorption spectrophotometer :

Atomic absorption spectro-photo-meter is based on the principle that when atomic vapours of an element are irradiated by the radiation of a characteristic wavelength (i.e. the light from a source whose emission lines are those of the element in question), they absorb in direct proportion to the concentration of the element being determined.

Instrument features :

A wide range of atomic absorption spectrophotometer is available today, all of them have the basic features in common and consist of the following components:

(a) A Light source :

A Light source emits the spectrum of the element to be determined. The most widely used light source is hollow cathode lamp which is

designed and operated in such a way that the lines to be measured are sharp, of stable intensity and free from background.

(b) Atomizer-Burner assembly :

A means of producing atomic vapours of the element to be analyzed. The solution to be analyzed is drawn by capillary and converted into stream of compressed air to a fine spray which after condensation of larger droplets is mixed with the fuel gas acetylene and burnt in a long flame (at 2100-2400^{o}C) in a stainless steel burner.

(c) A Monochromator :

It isolates the absorbing resonance lines from other non absorbing lines. When the light coming from the HCL, after traversing the flam, enters the monochromator which is already set at the wavelength of the resonance lines of the desired element, the monochromator performs its function.

(d) A Detector :

It measures the magnitude of absorption of the characteristic radiation.

(e) A Photomultiplier Tube :

It amplifies the absorption signal and converts the light radiation into electrical energy.

(f) A readout system :

It measures the absorbance in volts. It is normally a strip chart recorder, a digital display, a meter or printer. The presently available AAS have features like automatic calibration with one or more standards, automatic curve corrections, automatic and foolproof gas switching and calculation of average and standard deviations in repetitive runs.

Collection and preparation of soil and plant samples : To avoid contamination, soil samples are to be collected in plastic tub, using rust free instrument or wood and kept in polythene lined cloth bags. Samples are prepared with the help of wooden mortar and pestle and sieved through 2mm nylon screen/mosquito net cloth or stainless steel sieve.

Similarly plant samples (leaves, grains or straw) should be washed with 0.01N HCl, rinsed with glass distilled water dried in oven at 65°C and crushed with the help of stainless steel scissors.

Soil extraction : DTPA offers the most favorable combination of stability constants for the simultaneous complexing of Zn, Cu, Fe and Mn, Cd, Co, Ni and Pb (Lindsay and Norvell, 1978). Buffering of extractant in a slightly alkaline pH range (7.3) by including soluble Ca^{2+}, avoids the dissolution of $CaCO_3$ with the release of occluded micronutrients due to CO_2 partial pressure of approximately 10 times that in atmosphere, as the soil contains slightly higher CO_2 levels than found in the atmosphere.

(a) Extracting solution : (0.005 M DTPA) Dissolve 1.9679g of DTPA (Diethylene tri amine penta acetic acid) + 13.3 ml TEA (Triethanol amine) + 1.47g $CaCl_2$.$2H_2O$ in 200 ml distilled water, dilute to 900 ml, adjust pH 7.3 with 6N HCl while stirring and then make upto 1 liter and mix thoroughly.

(b) Apparatus required : Shaker (Horizontal or Rotatory), iodine value flasks (100 ml capacity) or conical flasks with glass stoppers, funnels, filter paper whatman No.1, plastic storage bottles and Atomic absorption spectrophotometer.

(c) Stock Standard Solutions : The standard solutions of different micro-nutrients should preferably be prepared by using their wires. Dissolve 1g wire in a minimum volume of 1:1 nitric acid and dilute to 1000ml with distilled water to obtain 1000 µg/ml solution of micro-nutrient, or take salts of metals as follows:

Zn- 4.398g l^{-1} $ZnSO_4$,$7H_2O$
Cu- 3.929g l^{-1} $CuSO_4$,$5H_2O$
Fe- 4.977g l^{-1} $FeSO_4$,$7H_2O$
Mn- 3.598g l^{-1} $MnSO_4$,H_2O.

The prepared standards are also available in the market. Out of these standards, prepare working solution of 50 ppm. Then a series of standard solution of 0.5, 1.0, 1.5, 2.0 and 2.5 ppm may be prepared for each metal.

(d) Background correction : The reading of a spectral line always includes any contribution from the flame and sample matrix. Failure to correct properly for the background reading can be a source of serious error. Although the need for fast background correction is most obvious with graphite furnace work, it is also a consideration with flame atomic absorption.

The most common method of background correction in atomic absorption spectrometry involves the use of a continuum source such as a deuterium lamp to measure the background. The

source used is a deuterium filled discharge lamp, which emits an intense continuum spectrum from 190 nm to about 400 nm. This is the region where most atomic absorption lines occur and where the effects of background absorption are most pronounced. The poly-atomic gas D_2, is used in the lamp because a continuum is produced rather than a line spectrum.

The deuterium lamp is different from a hollow cathode lamp in construction and operation. The lamp incorporates a heated, electron-emitting cathode, a metal anode and a restrictive aperture between the two. A discharge current of several hundred milli amperes excites the deuterium gas. The discharge is forced to pass through the small aperture, forming a defined area of high excitation and hence high light emission. A suitable window transmits the light to the spectrometer's optical system.

To obtain successful background correction the deuterium lamp must be correctly aligned, and its intensity must be matched to that of the hollow cathode lamp.

It is important that both the deuterium source and the hollow cathode source are aligned to follow the same optical path. If they are not, then the two measurements may not be made on the same atom population and significant errors may occur.

In order to balance the intensity of the deuterium lamp with the hollow cathode lamp, it may be necessary to change the hollow cathode lamp current to a higher or lower value depending on the relative intensities of the lamps.

Although most modern AA spectro-photometers incorporate so called "simultaneous" background correction, they rely on two measurements separated slight in time. One measurement is of the total absorbance (atomic plus background) and the other is of the background only. The background is electronically subtracted from the absorbance to give the background corrected atomic absorbance with the continuum source method of background correction, the total absorbance is measured during the hollow cathode lamp pulse and the background during the deuterium lamp pulse. With the Zeeman method using a modulated magnetic field, the total absorbance is measured with the magnetic field off and the background with the field on.

(e) Soil analysis : Weigh 12.5g soil sample in 100 ml iodine value flasks. Add 25 ml DTPA solution. Shake this mixture for 2 hours on shaker at 70 to 80 oscillation per minute, filter through acid washed distilled water rinsed, whatman No.1 filter paper and collect the filtrate in plastic bottles. Determine the content of micronutrients on atomic absorption spectrophotometer.

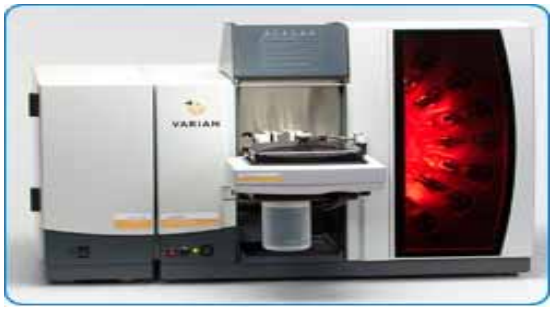
(i) Plant analysis : Weigh 0.5g plant sample in a conical flask (corning, 100 ml capacity). Add 10 to 12 ml of di acid mixture (1 perchloric + 4 nitric acid) and digest the mixture on hot plate till the residue is colour less. Now take off, cool dilute with distilled water and filter through whatman No.1 filter paper. Make up the volume of digestate to 50 ml. Read for micronutrient content on atomic absorption spectro-photometer.

Factors : For soil multiply the concentration read on AAS computer sheet by "2". Similarly for plants the multiplying factor will be 100 to get concentration in mg kg^{-1}.

Reference :

Lindsay, W.L. and Norvell, W.A. (1978). Proc. Soil Sci. Soc. Am. 42 : 421-428.

Atomic Absorption Spectrophotometer



Estimation of Boron in Soils, Plants and Water

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Boron occurs as anion in soils and is required by plants in very small quantity. Water soluble B makes the estimate of its availability to plants. Total boron in soils varies from 20 to 200 mg kg^{-1} and available (water soluble) boron in soils ranges from 0.03 to 12 mg kg^{-1} respectively. The threshold value ranging from 0.1 to 0.5 mg kg^{-1} (water soluble B) depends upon the soil type, crops, and other factors, below which the response to applied boron may be expected. Some sensitive crops to boron deficiency are listed in table 1. Its availability is affected by soil pH as under:

- Deficiency of B is generally observed in old acid leached soils.
- Availability increased with the rise in soil pH having significant positive correlation with pH rising from 4.7 to 6.7.
- In neutral, saline and calcareous soils the B availability again decreases with the rise in soil pH having significant negative correlation with the rise in pH from 7.1 to 8.1. In calcareous soils B fixation occurs with the condensation of borate radical into long chains in the presence of Ca.

Table 1 : Sensitivity of crop to B deficiency

Sensitive	Medium	Low
Alfalfa	Apple	Barley
Cauliflower	Cabbage	Beans
Rape seed	Carrot	Corn
Conifers	Clover	Grasses
Peanuts	Cotton	Oat
Sugarbeet		Onion
Turnip		Pea
		Potato
		Soybean
		Wheat
		Rice

- In alkaline soils the availability of B is high and may be even toxic for plant growth.

Besides this the low moisture availability also causes B deficiency.

Irrigation water containing Boron between 0.3 to 0.6 mg kg^{-1} can be used safely, whereas, irrigating soils with water containing 1 to 3 mg kg^{-1} B causes toxicity of B in plants.

Boron determination (Azomethine H Method) :

Azomethine H forms coloured complex with H_3BO_3 in aqueous media. Over a concentration range of 0.5 to 10 µg B/ml the complex is stable at pH 5.1. Maximum absorbance occur at 420 nm with little or no interference from a wide variety of salts. This technique is rapid, reliable and more convenient to use than traditional procedures employing carmin, curcumin or quinalizarin (John *et al*., 1975).

Apparatus:

(1) Spectrophotometer
(2) Poly-propylene tubes 10 ml capacity.

Reagents :

1. Distilled water
2. Buffer solution : Dissolve 250 g of ammonium acetate (NH_4OAc) and 15 g of ethylene diamine tetra acetic acid (EDTA disodium salt) in 400 ml of distilled water. Slowly add 125 ml of glacial acetic acid and mix.
3. Azomethine H reagent : Dissolve 0.45 g of azomethine H in 100 ml of 1% L ascorbic acid solution. Fresh reagent should be prepared weekly and stored in a refrigerator.
4. Calcium hydroxide suspension : Add 0.4g $Ca(OH)_2$ to 100 ml distilled water.
5. 0.1 N HCl : Add 8.3 ml conc. HCl to 900 ml distilled water, mix, cool to room temperature and make up the volume to 1000 ml.
6. Calcium chloride 0.01 M Dissolve 1.11 g of anhydrous $CaCl_2$ in 900 ml distilled water and make up the volume to 1000 ml.
7. Boron standard solution : Dissolve 0.114g of Boric acid (H_3BO_3) in distilled water and adjust the volume to 1000 ml. Each ml

contains 20 µg B. Dilute 10, 20, 30, 40 and 50ml of the stock solution to 100 ml with distilled water to have solution with B concentration of 2,4,6,8 and 10 µg of B/ml respectively. Include a distilled water sample for the 0.0 µg of B/ml standard solution.

Procedure :

Take 1 ml of aliquot of blank and diluted B standards into a 10 ml polypropylene tube, add 2 ml of buffer solution and mix. Add 2 ml of azomethine H reagent, mix and after 30 minutes read the absorbance at 420 nm on spetrophotometer. With the help of absorbance readings of standard solutions of different concentration of B the standard curve is drawn and a factor for concentration of B for 1 absorbance is calculated which is utilized to calculate B in the soils, plant or water sample.

Preparation of Extracts :

1. **Soil extracts** : The hot water soluble extraction procedure of Berger and Truog (1939) is being used widely with slight modification of adding dilute electrolyte (0.01 M $CaCl_2$) instead of water only. This provides clear, colourless extract which eliminates the need of charcoal for decolourzation. Beside this a negative error, associated with B adsorption by charcoal, is also removed.

Place 20 g air dry soil in 250 ml low B flat bottom flasks and add 40 ml of 0.01 M $CaCl_2$ solution. Attach water cooled reflux condenser to the flask. Heat the flasks for 5 minutes and then cool and filter the suspension in plastic bottles.

Transfer 20 ml aliquot to evaporating dish, add 2 ml $Ca(OH)_2$) suspension and evaporate the solution to dryness. Heat the evaporating dishes gently to destroy organic matter, cool to room temperature, add 5 ml 0.1N HCl. Triturate the residue with rubber policeman to ensure the complete dissolution of the residue (Bingham, 1982).

For analysis of B pipette 1 ml of the aliquot and proceed as for the standard curve.

2. **Plant digest** : Take 0.5 g plant sample in porcelain/platinum dishes Add 0.5 g $Ca(OH)_2$. Ignite the sample in the muffle furnace at 550°C for 4 hours to obtain white grey ash. Cool the dishes and moist the ash carefully with little distilled water and then add 5 ml 0.1N HCl. Transfer the content in to 25 ml volumetric flask mix and make up the volume to 25 ml with distilled water. For analysis of B take 1 ml of the aliquot and proceed as for the standard curve.

3. **Water analysis** : Take suitable quantity of water sample (containing 0.2 to 5.0 µg B) in porcelain dishes add 2 ml $Ca(OH)_2$ and proceed as described for soil extract. It is important to keep a definite volume of aliquot i.e. 1 ml of either soil, plant or water in final step of B determination.

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Estimation of Microbial Biomass Carbon in Soil

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Microbial biomass carbon :

It was estimated by chloroform fumigation extraction method (Brookes *et al.*, 1985).

Principle:

Overnight fumigation of chloroform is done to kill all the organisms in soil samples; after which the amount of the organic C in the sample can be measured by fumigation- extraction method.

Fumigation extraction method:-

The microbial biomass constituents released by $CHCl_3$ fumigation treatment can be extracted directly through chemical extractants and the readily oxidisable C contained in the extract can be measured through standard chemical procedures.

Reagents

1. Ethanol free chloroform
2. Conc. H_2SO_4
3. 0.5M K_2SO_4**:** 43.563 g K_2SO_4 was dissolved in distilled water and diluted with 500 ml.
4. 0.2 N $K_2Cr_2O_7$**:** 0.9808 g $K_2Cr_2O_7$ was dissolved in 100 ml of distilled water.
5. Orthophosphoric acid
6. 0.005 N Ferrous Ammonium Sulphate (FAS): 3.92 g of ferrous ammonium sulphate was dissolved in 0.15 ml conc. H_2SO_4 and then diluted to 2 liters by distilled water.
7. Ferroin/Diphenylamine indictor

Procedure

1. 10g of soil (three sets of each soil sample) was weighed and kept in to 100 ml beakers each.
2. 8 ml of distilled water was added to both beakers and were incubated for seven days at 25^0C in an incubator.
3. 20 ml of chloroform was taken in a separating funnel. It was washed two times with conc. H_2SO_4 (with half of the volume of chloroform) and the acid (bottom phase) was discarded. It was washed twice with the same volume of distilled water similarly to make the chloroform free of ethanol and the bottom whitish phase was collected.
4. One set of soil samples were taken in crucible and fumigated with ethanol free chloroform in a vacuum desiccator. For the purpose 20 ml ethanol free chloroform was taken in Petridis and was placed inside the desiccator the bottom portion inside the vacuum desiccators. It was attached to the vacuum pump and the air was evacuated until the chloroform starts boiling to saturate the desiccators with chloroform fumes. Then the vacuum desiccator was kept in dark room for overnight.
5. Next day the vacuum was released and chloroform was removed from the desiccator.
6. 10 g each of fumigated and non fumigated soil samples were weighed in 150 ml conical flask and 40 ml of K_2SO_4 (0.5 M) was added to each flask. Samples were shaken for 30 minutes on a rotary shaker.
7. Both the samples were filtered with Whatman no. 42 filter paper, labelled and freezed until digestion.
8. 10 ml of the filtrate was taken in 100 ml conical flask and 2 M of $K_2Cr_2O_7$ (0.2 N) and 10 ml of con. H_2SO_4 was added to it. The contents of the flask were allowed to cool for half an hour then 5 ml orthophosphoric acid was added along with 200 ml distilled water. Minimum two blanks were also run with 10 ml distilled water.
9. Few drops of diphenylamine indicator were added and titrated against ferrous ammonium sulphate (0.2 M) till the colour changed from violet to green.

Calculation:

$$\text{Microbial carbon (ppm)} = \frac{\text{Fumigated C} - \text{Unfumigated C}}{0.44}$$

Reference:

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Gamma Irradiation and its Importance for Food Preservation

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Gamma irradiation has been extensively used for food irradiation and sterilisation, killing of fungus and micro organisms, sterilisation of medical accessories and surgical equipments, high energy radiation chemistry, seed irradiation and semiconductor irradiation. Gamma Chamber can also be used in many other research applications which require irradiation of materials with ionizing radiations to varying doses.

The radiation processing of food involve the controlled application of energy from ionizing radiation such as gamma rays, electrons and X rays for food preservation. The gamma rays and X-rays are short wavelength radiation of electromagnetic spectrum, which includes radio waves, microwaves, infrared, visible, and a violet light. Radioisotopes such as cobalt 60 and caesium-137 emit the gamma rays, while machines using electricity generate electrons and X-rays. The gamma rays and electrons are distinguished from other form of radiation by their ionizing ability. (That they are able to break chemical bond when absorbed by material). The product of ionizing radiation may be electrically charged ions) or neutral (free radicals). These there further react to cause change in an irradiated material known as the process of radiolysis. It is this reaction that causes the death of micro- organism, insect and parasites during food irradiation.

The conservation and preservation of food is a prerequisite for food security. It provides self-reliance to nation. The Indian Food Industries contributes about 25-28% towards GDP. The food processing sector provides 60-65% employment with a turnover of in US$ 36.1 billion of which US$ 27.8 billion in organized sector, any change or any stagnation in technology will inevitably have very large impact throughout the economy. India is a potential producer of fruits and vegetables live stock and marine products. India has a tremendous potential as the world largest food factory.

It has been estimated that about 30-35% of fruit and vegetables of worth Rs 3000/- corers are perished every year. The reasons for such losses are seasonal nature of fruits and vegetables production. The long distance between production and consumption centers and also rising gap between demand and supply. The hot and humid climate in the country is also quite favorable for the growth of numerous insects and micro organisms that destroy stored crops and cause spoilage of food every year. The spoilage also occurs due to chemical and physiological changes in stored foods. To preserve the food and food products, technologies such as freezing caning sun drying pickling fermentation have been recommended by researchers but, each of these methods have its own merits and limitation. The search for an alternative newer economical methods to preserve food and causes least changes in sensory quality have been under taken since long back, and has been observed that radiation processing of food is one of the latest method developed for food preservation.

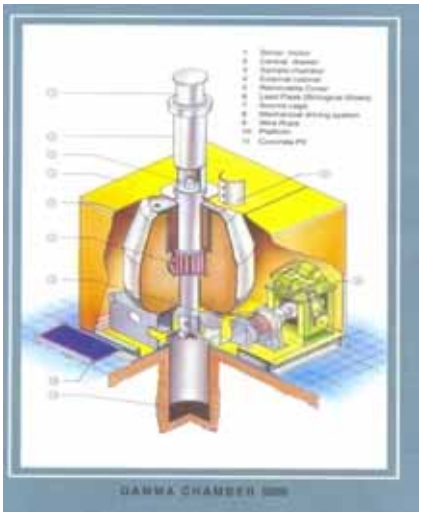
Irradiation by Gamma Chamber 5000 :

Gamma Chamber 5000 is a compact self shielded cobalt-60 gamma irradiator providing an irradiation volume of approximately 5000cc. The material for irradiation is placed in an irradiation chamber located in the vertical drawer inside the lead flask. This drawer can be moved up and down with the help of a system of motorized drive which enables precise positioning of the irradiation chamber at the centre of the radiation field.

Radiation field is provided by a set of stationary cobalt-60 sources placed in a cylindrical cage. The sources are doubly encapsulated in corrosion resistant stainless steel pencils and are tested in accordance with international standards. Two access holes of 8 mm diameter are provided in the vertical drawer for introduction of service sleeves for gases, thermocouple, etc. A mechanism for rotating/stirring samples during irradiation is also incorporated. The lead shield provided around the source is adequate to keep the external radiation field well within permissible limits. The Gamma

Chamber 5000 unit can be installed in a room measuring 4 metres x 4 metres x 4 metres.

GAMMA CHAMBER 5000



Features :

- **Safe and self-shielded:** The shielding provided is adequate to limit the radiation field on the external surface of the unit, well within the permissible levels. No additional shielding is required for its installation and use.
- **Automatic control of irradiation time:** Built-in timer provides accurate control of irradiation time from 6 seconds onwards. The unit can also be operated manually. Solid state programmable controls have been provided. In the event of power failure battery backup displays the programmes.
- **Manual control of irradiation temperature:** It is possible to irradiate samples at low or high temperature by circulating liquid nitrogen or hot air. These can be introduced through the service sleeves provided in the vertical drawer. The irradiation temperature is sensed by a thermocouple and displayed on the panel.
- **Remote operation:** An additional table top control panel is provided for remote operation in addition to the normal one provided on the unit.
- **Dose uniformity:** Stationary source pencils, symmetrically placed in a cylindrical cage ensure good uniformity of radiation field in the sample chamber. In addition a mechanism is also provided for rotating/stirring samples during irradiation.
- **Easy loading and unloading of samples:** Sample chamber extends to a convenient height for easy loading and unloading of samples.
- **Safety assurance:** The design of Gamm Chamber conforms to American National Standards, ANSI-N433.1-1977 for safe design and use of self-contained dry source storage gamma irradiators (Category I). It also meets the requirements of type B(U) package for safety in transport of radioactive materials as per AERB code No.SC/TR-1, 1986 of Atomic Energy Regulatory Board of INDIA.

Applications :

Gamma Chamber is a versatile equipment for research studies in many fields such as:

- Radiobiology
- Preservation of tissue grafts
- Mutation breeding
- Food preservation
- Sterile male insect technique
- Biological and genetic effects of radiations
- Radiation chemistry
- Radiation effects on materials

- Radiation sterilization
- Modification of properties of materials

Food Preservation by Gamma Radiation

The radiation processing of food is carried out inside an irradiation chamber shielded

by 1.5 - 1.8 meter thick concrete walls. Food either pre-packed or in bulk placed in suitable containers is sent into the irradiation chamber with the help of an automatic conveyor. The conveyor goes through a concrete wall labyrinth, which prevents radiation from reaching the work area and operator room. When the facility is not in use the radiation source is stored under 6 meter deep water. The water shield does not allow radiation to escape in to the irradiation chamber, thus permitting free access to personnel to carry out plant maintenance. For treating food, the source is brought to the irradiation position above the water level after activation of all safety devices. The goods in aluminum carriers or tote boxes are mechanically positioned around the source rack and are turned round their own axis, so that contents are irradiated on both the sides. The absorbed dose is determined by the residence time of the carrier or tote box in irradiation position.

Measurement of radiation dose :

Placing dosimeters at various positions in a tote box or carrier we can check the absorbed dose. The dosimeters are made from a material including photographic film, Perspex and cobalt glasses. The poly vinyl chloride (PVC) dosimeters are impregnated with a dye. The Hydrogen chloride is released from the PVC by irradiation and it produces a qualitative or quantitative change in the colour of the dye to indicate the dose received.

Dose distribution :

The penetration of gamma radiation depends on the density of the food as well as the energy of the ray. At a density of 1000 kg m^{-3} half of the rays are absorbed in 11 cm. Halving the density approximately double the depth of penetration. The uniformity of dose distribution can be expressed as a ratio of D max : D min. For sensitive food such as chicken the ratio should be as low as possible1.5.

Potential Applications of Gamma Radiation:

The radiation dose administrated to a food depends on the resistance of the organisms present and the objective of the treatment. The maximum recommended dose is 15 kGy, with average dose not exceeding 100 kGy. Various application of this technology are as under:

1. **Sterilization (or radappertisation) :**

   It is possible to sterilize meat and other product, the dose required exceed the current limit of 10 kGy. A dose of 48 kGy is needed for 12 D reduction of *Cl. butulinum*. High dose makes the product organoleptically un acceptable.

2. **Reduction of pathogens (radicidation):**

   Food poisoning bacteria such as *salmonella typhimurium* are less resistant to radiation than *Cl. Botulinum*, and doses of 3-10 kGy are sufficient for destruction.

3. **Prolonging shelf life (or radurisation) :**

   Relatively low doses are needed to destroy yeast, moulds and non-spore forming bacteria. This process is used to increase shelf life by an overall reduction of vegetative cells.

Table 1: List of radiation processing facilities available in the world :

S. No.	Country	No. of irradiators	Food Commodities
1.	Algeria	1	Potato
2.	Argentina	1	Spices, spinach, cocoa powder
3.	Bangladesh	1	Spices, onion, dried fish
4.	Belgium	1	Spices, dehydrasted vegetables, deep frozen foods
5.	Brazil	3	Spices, dehydrasted vegetables, fruits, vegetables, grain
6.	Canada	1	Spices
7.	Chile	1	Spices, dehydrasted vegetables, onion, potato, poultry meat
8.	China	11	Spices and vegetable seasonings, Chinese sausage, garlic, apple, potato, onion, dehydrated vegetables, souses, rice, tomatoes
9.	Croatia	1	Spices, food ingredients, dried beef noodles
10.	Czech. Repulic	1	Spices, dry food ingredients
11.	Cuba	1	Potato, onion, beans
12.	Denmark	1	Spices
13.	Finland	1	Spices
14.	France	5	Spices, vegetable seasonings, herbs, poultry (frozen boneless chicken, dried fruit, frozen frog legs, shrimp)

15.	Hungary	1	Spices, onion, wine cork, enzyme
16.	India	2	Spices, onion, potato
17.	Indonesia	2	Spices, rice
18.	Iran	1	Spices,
19.	Israel	1	Spices, condiments, dry ingredients
20.	Japan	1	Potato
21.	Korea Republic	1	Garlic powder, spices, condiments
22.	Mexico	1	Spices, dry food ingredients
23.	Netherland	1	Spices, frozen products, poultry dehydrated vegetables, egg powder, packaging material
24.	Norway	1	Spices
25.	Poland	3	Not specified
26.	Peru	1	Spices, food additives, animal feed
27.	South Africa	4	Spices, shelf-stable food, fruits
28.	Thailand	1	Spices, fermented pork sausages, enzymes
29.	Ukraine	1	Grain
30.	UK	1	Spices
31.	USA	10	Spices, poultry, fruits, vegetables
32.	Vietnam	1	Onion
33.	Yugosla	1	Spices
(Source : ICGFI, Food & Environmental Protection Section, Update, 1997)			

4. Control of ripening :

Fruits and vegetables can be irradiated to extend their shelf life about 2-3 time when stored at 10^0C The ripening and maturation of fruits are arrested by inhibition of hormone production and interrupting the biochemical process of cell division.

5. Disinfestations :

Grain and tropical fruits may be infested with insect and larvae, they reduces export potential. A low dose below 1 kGy is effective for disinfestations

6. Inhibition of Sprouting:

The technology is effective in inhibiting sprouting of potatoes, A dose of about 150 Gy has been recommended. Similar doses are also effective in preventing sprouting of onion and garlic.

Benefits and limitation of gamma radiation processing:

Benefits :

1. Radiation processing is a cold process and therefore, unlike heat, it can be used on agricultural commodities without changing their fresh-like character
2. Radiation processing does not alter significantly nutritional value, flavour, texture and appearance of food
3. Radiation using Cobalt-60 cannot induce any radioactivity in food and does not leave any harmful or toxic radioactive residues on foods as is the case with chemical fumigants
4. Due to the highly penetrating nature of the radiation energy, it is a very effective method.
5. Prepackaged foods can be treated for hygienization and improving shelf-life
6. The radiation processing facilities are environment friendly and are safe to workers and public around.

Limitations:

1. Radiation processing is a need based technology and cannot be applied to all kinds of foods
2. Radiation processing cannot make a bad or spoiled food look good
3. It cannot destroy already present pesticides and toxins in foods
4. Amenability of a particular food commodity to radiation processing has to be tested in a laboratory.

Plant Mutation Breeding by Gamma Radiation

Plant mutation breeding by radiation has been investigated for long time in many countries. New mutant varieties give us useful gene resources for the security of food resources, the conservation of our ecosystem, and the promotion of new industries. Using radiation technique (gamma-rays, X-rays and EB) 128 varieties were developed in Japan. Many new species were developed for disease resistant crops, i.e. 55 species of rice, 10 of barley and 2 of wheat. Other species of beans, fruits including pears resistant for black spot decease, grass, vegetables, etc, were also developed.

Recently, a lot of fascinating new mutants are generated by ion beams. Ion beams can frequently cause large DNA alterations such as inversion, translocation and large deletion rather than point mutation, which result in producing characteristic mutants otherwise attainable. Ion-beam irradiation of *Arabidopsis* seeds has produced the UV-B-resistant, the frilled-petal, and other novel mutants. The features of ion beams in the mutation induction seem 1) to induce mutants with high frequency, 2) to show a broad mutation spectrum, and therefore, 3) to produce novel mutants. New mutants of chrysanthemum and carnation with complex and striped flower-color, and new flower-shape have been produced and commercialized.

Nuclear techniques, in contrast to conventional breeding techniques, are widely applied in agriculture for improving genetically diversity. Unlike conventional breeding procedures which involve, the production of new genetic combinations from already existing parental genes, nuclear technology causes exclusively new gene combinations with high mutation frequency. Basic tool of nuclear technology for crop improvement is the use of ionizing radiation which causes induced mutations in plants. These mutations might be beneficial and have higher economical values.

Measures of activity (A) :

The number of disintegrations, or decay events, or nuclear transformations, in a sample per unit time is its activity A. Two common informal units are disintegrations per second and disintegrations per minute.

Curie (Ci) : The US unit of activity is the curie (Ci).$3.7x10^{10}$ disintegrations per second. Common multiples are the millicurie and microcurie.

Becquerel (Bq) : The SI unit of activity is the becquerel (Bq). One becquerel is 1 disintegration per second. The common multiple is the megabecquerel (1 mCi = 37 MBq).

Half –life: The time (t) taken for the radioactivity of a sample to fall to half its initial value.

$\mathbf{t}_{1/2}= 0.693 / k$

Electron volt (eV): energy of radiation (usually as mega electron volts (MeV). $leV=1.602x\ 10^{-19}$ J

Grays (Gy): Absorbed dose (where 1 Gy is the absorption of 1 J of energy per kilogram of food)
Previously rods (radiological unit) were used. 1 rad = 10^{-2} J kg^{-1}

Determination of Soil Aggregates

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Soil aggregates refer to a group of two or more primary particles which cohere to each other more strongly than to surrounding particles. Under field conditions, all such units also cohere to some degree with neighboring aggregates and form larger aggregates. Such aggregates form and break easily into smaller aggregates during tillage and by disruptive action of water and air. The aggregates maintaining their identity are those in which the cohesive forces among particles were greater than the disruptive forces. A quantitative characterization of aggregates is done by determining aggregate stability and size distribution of aggregates. The aggregate stability refers to the resistance of soil aggregates to breakdown by water, air, and mechanical manipulations. Tillage operations at lower or higher water contents greatly decrease the aggregates stability and size distribution of aggregates in a soil. The size distribution of wet and dry aggregates determines overall tilth, size of pores and susceptibility of aggregates to movement by water and wind.

1. Water Stable Aggregates

The soil aggregates which can stand the disruptive forces of wetting by water are known as water stable aggregates. Therefore, the processes of wetting, disruption of dry aggregates and screening of aggregates of different sizes should reasonably compare to the disruptive action of water and mechanical forces of tillage under wetland conditions. The vacuum wetting of dry soil largely simulates the process of soil wetting in the subsurface layers but the immersion wetting is more comparable to the wetting of surface soil by irrigation. Similarly, sieving under water compares more closely with the disruptive action of water and other mechanical forces as experienced in wetland rice field preparation. The mean weight diameter (MWD) of water stable aggregates is a useful index to characterize the change in structure due to puddling. The MWD is calculated as

$$MWD = \sum_{i=1}^{n} \bar{x}_i w_i$$

Where $\bar{x}_i$ = mean diameter of size fraction i, and w_i = proportion of the total sample weight occurring in the corresponding size fraction i. The summation is carried over all n size fractions including the one that passes through the finest sieve.

Apparatus and accessories

Standard sieves of diameter 12.7 cm and height 5 cm and hole of 8.0, 5.0, 1.0, 0.5, 0.25 and 0.1 mm (2 sets), Yoder apparatus, which raises and lowers the nests of sieves through water 3.8 cm, approximately 30 times per minute, atomizer, Physical balance, 105 ^{0}C oven, desiccator, 8 cm watch glasses, hydrogen peroxide, and 0.1 N hydrochloric acid.

Procedure

1. Take 200 g of air-dry natural soil which can pass through 8.0 mm screen and retained on 5.0 mm screen. Do not break them by hammer. Large gravels or roots should be removed.
2. Weigh 25 g aggregates of 5.0-8.0 mm size in three watch glasses. Keep one of them in oven at 105 ^{0}C for moisture determination and use the other two for analysis in duplicate.
3. Arrange two sets of six sieves in the order of 5.0, 2.0, 1.0, 0.5, 0.25 and 0.1 mm from top to bottom.
4. Spread the sample (25 g) of aggregates evenly over top 5 mm sieve and spray 5 to 10 ml of salt-free water on them. Wait for 3 to 5 minutes and spray another 5 to 10 ml of water.
5. Transfer the nest of sieves to the drum of the sieve shaker and clamp them in position. Fill the drum, with salt-free water up to a level slightly below the top screen when sieves are in highest position. Turn the pulley of the shaker slowly by hand to attain the highest position.
6. Lower the sieves to the lowest position and wet the aggregates for 10 minutes. Fill more water in the drum so that aggregates are just covered with water when sieves are again in highest position.
7. Switch on the oscillator and let the sieves oscillate in water for 10 minutes with a frequency of 30 cycles per minute through a stroke length of about 3.8 cm.
8. Take out the nest of sieves, let the water drain, separate and place them on paper sheets, and let the aggregates on each sieve dry and harden in air.

9. Transfer the aggregates of each sieve in to separate moisture boxes. Find out their oven-dry weight.

10. Transfer the aggregates from the moisture boxes into 250 ml beakers separately and oxidize and disperse using H_2O_2 and sodium hexametaphosphate respectively. Use mechanical stirrer for complete dispersion; pass the dispersed aggregates through the same sieves on which they were retained originally. Collect the unaggregated primary particles from each sieve and record their oven-dry weight. Subtract the weight of the primary particles from the weight of the aggregates obtained on respective sieves in step-9.

11. Calculate the percentage of aggregated soil particles on different sieves.

12. Plot a graph between accumulated percentage of soil remaining on each sieve as ordinate and upper limit of each size fraction as abscissa.

$$H.\ \text{Mean Weight Diameter} = \sum_{i=1}^{n} \bar{x}\, w_i \ \text{mm}.$$

Where n= 6, the number of size fractions.

Mean weight diameter of aggregates (mm)	I	II

Results

1. Percent aggregates greater than 0.1 mm (mean of 2 observations) =

2.Mean weight diameter (mean of two observations) mm =

13.Calculate the mean weight diameter (MWD) of aggregates in mm and report the results as MWD and percent aggregation.

Observations

		I	II
A. Air dry weight of sample (g)	=	25	25
B. Moisture percent in soil (%)	=		
C. Frequency of oscillation (min^{-1})	=		
D. Stroke length (mm)	=		
E. Oven-dry weight of particles: a) aggregated (g):	=		
b) Unaggregated (g):	=		
F. Oven-dry weight of sample (g)	=		

Calculation

S. No.	Size Range of aggregates (mm)	Weight of particles retained on the sieve (g)				Wt. of aggregated particles		% aggregation		Accumulated percentage	
		Before dispersion		After dispersion		I Sample	II Sample	I Sample	II Sample	I Sample	II Sample
		I Sample	II Sample	I Sample	II Sample	c-d		(e x100)/ F			
a	b	c		d		e		f		g	
1.	8.0-5.0										
2.	5.0-2.0										
3.	2.0-1.0										
4.	1.0-0.5										
5.	0.5-0.25										
6.	0.25-0.10										

Determination of Bulk Density of Soil by Core Method

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Density is an expression of mass of the oven-dry soil per unit volume. Two types of densities are recognized - the bulk density and the particle density. In bulk density total soil volume includes the volume of solids and pore spaces, whereas in particle density the volume of solids alone is considered.

Bulk Density: Bulk density is the ratio of oven-dry mass of soil to its total volume before drying. Knowledge of bulk density is useful in calculating moisture content by volume, porosity using particle density, volume of a known weight of soil, and weight of a furrow-slice. Bulk density is a direct measure of soil compaction.

Bulk density is highly dependent on any kind of physical manipulation, degree of wetness, and kind and arrangement of soil particles. For this reason, preservation of original structure in the sample is most essential aspect of the methodology for determination of bulk density. Common methods of determining soil bulk density use soil cores or clods in their natural state.

Principle: The core method involves pressing uniformly a metal core sampler of 0.05 to 0.10 m diameter into the soil to a desired depth and removing carefully. The sample is dried to constant weight at 105 ^{0}C and weighed, and the bulk density is expressed as oven-dry mass divided by the field volume of the sample. The core method is unsuitable in stony soils.

Apparatus and Accessories: Core sampler with removable sample cylinder, shovel, straight edged knife, moisture box, cellophane tape, balance and oven.

Procedure

1. Select two grass-free, and crack-free smooth soil surface for duplicate sampling.
2. Apply a heavy lubricant on the inside wall of the core sample cylinder.
3. Place the core sampler over the selected spots and insert the core in to the soil at desired depth by hammering on the top of the handle.
4. Tilt the sampler a little forward and backward to partly detach from the soil mass below and carefully lift the sample without any jerk and disturbance of natural structure. In certain soil fractions, a shovel may be needed to cut from sides and under the sampler to remove the sampler without disturbance.
5. Unscrew the lid over the outer core cylinder and carefully remove the sample holder.
6. Trim the excess soil from each end of the sample holder with a straight-edged knife so that the sample volume is the same as the volume of the sample holder.
7. Transfer the sample to a moisture box and seal it for transportation to the laboratory.
8. Draw samples in duplicate from the desired depths.
9. In the laboratory, remove the seal, weigh the sample, uncap, and place in oven at 105 ^{0}C for drying until constant weight.
10. Report bulk density along with moisture content in the sample.

Observations

A. Date ----------
B. Field number or name----------
C. Present crop ----------, previous c
D. Volume of the sample holder ----------
Calculation and Results

$$\text{A. Bulk density} = \frac{\text{oven dry weight of soil sample}}{\text{volume of sample}}$$

= 1. ----------, 2. ----------, Av. ----------g/ cm^3

B. Water content =.....

S.No.	*Sampling depth (m)*	*Weight of moisture box (g)*	*Weight of moisture box + fresh sample (g)*	*Weight of moisture box + dry sample (g)*	*Oven dry wt. Of soil (g)*	*Water content in the sample (g/g)*
1.						
2.						

Estimation of Ascorbic Acid in Vegetables and Fruits

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Ascorbic acid (vitamin C) is a sugar acid and an antiascobutic. It is required for normal formation of connective tissue collagen, specifically for hydroxylation of certain proline and lysine residues. It is also required for normal iron metabolism and a strong reducing agent losing hydrogen atom readily to become dehydroascorbic acid which has also vitamin C. Ascorbic acid is water soluble, heat labile vitamin and acts as an antioxidant to protect the cell membrane from the toxic action of powerful oxidizing agents. Generally it is present in all fresh vegetables and fruits, found abundantly in berries, citrus, guavas, chillies and green leafy vegetables. Estimation of ascorbic acid content in fruits and vegetables by volumetric method is described below:

Principle:

Ascorbic acid reduces the 2, 6-dichlorophenol indophenols dye to a colourless leuco-base. The ascorbic acid gets oxidized to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is the appearance of pink colour. The dye is pink coloured in acid medium. Oxalic acid is used as the titrating medium.

L-Ascorbic acid + 2, 6-Dichlorophenol indophenol (oxidized)

⟶ 2, 6-Dichlorophenol indophenols (reduced)

+Dehydroascorbic acid

Equipments and glass wares:

Balance, Reagent bottle, Pestle, Mortar, Test tube, Centrifuge, Volumetric flask, Conical flask, Funnel and Burette.

Reagents:

1. **Oxalic acid (4 %)**
2. **Dye solution:** weight 42 mg sodium bicarbonate into a small volume of distilled water. Dissolve 52 mg 2, 6-dichlorophenol indophenol in it and make up to 200 ml with distilled water.
3. **Stock standard solution:** Dissolve 100 mg ascorbic acid in 100 ml of 4% oxalic acid solution in a volumetric flask. The concentration of stock standard solution is 1 mg ml^{-1}.
4. **Working standard**: Dilute 10 ml of the stock solution to 100 ml with 4% oxalic acid. The concentration of working standard is 100 µg ml^{-1}.

Procedure:

1. Weigh 0.5-5 g fresh sample kept in a mortar, crush the sample with 4% oxalic acid using a pestle and make up to a known volume of extract (100 ml) and filter or centrifuge.
2. Pipette out 5 ml supernatant or aliquot into conical flask, add 10 ml of 4% oxalic acid and titrate against the standard dye solution (V_2 ml) to end point appearance of pink colour.
3. Repeat the procedure with a blank solution omitting the sample.
4. Similarly, pipette out 5 ml ascorbic acid working standard solution into 100 ml conical flask, add 10 ml of 4% oxalic acid and titrate against the standard dye solution (V_1 ml).
5. The end point is the appearance of pink colour which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid.
6. Calculate the ascorbic acid content of fresh sample of tissue as mg per 100 g.

Calculation:

Amount of ascorbic acid mg 100 g^{-1} sample =

$$\frac{0.5 \text{ mg}}{V_1 \text{ ml}} \times \frac{V_2}{5 \text{ ml}} \times \frac{100 \text{ ml}}{\text{Wt. of the sample}} \times 100$$

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Estimation of Chlorophylls in Vegetables and Fruits

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Chlorophylls are the essential components for photosynthesis, and occur in chloroplasts as green pigments in all photosynthetic plant tissues. They are bound loosely to proteins but are readily extracted in organic solvents such as acetone or ether. Chemically, each chlorophyll molecule contains a porphyrin (tetrapyrole) nucleus with a chelated magnesium atom at the centre and a long-chain hydrocarbon (phytol) side chain attached through a carboxylic acid group. There are at least five types of chlorophylls in plants. Chlorophylls *a* and *b* occur in higher plants, ferns and mosses. Chlorophylls *c*, *d* and *e* are only found in algae and in certain bacteria. Estimation of chlorophylls content in vegetable and fruit plant materials spectrophotometrically and their method as describe below:

Principle:

Chlorophyll is extracted in 80 % acetone and the absorbances at 663 nm and 645 nm are read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll is calculated.

Equipments and glass wares:

Balance, Spectrophotometer, Centrifuge, Mortar, Pestle, Buchner funnel, Filter paper and Volumetric flask.

Reagents:

1. Analytical grade 80 % acetone (prechilled)

Procedure:

1. Weigh1g of finally cut and well mixed fresh sample of leaf or fruit tissue into a clean mortar.
2. Crush or grind the tissue with the help of pestle to a fine pulp with the addition of 20 ml of 80 % acetone.
3. Filter the extract of residue with a Buchner funnel through Whatman No. 42 filter paper or centrifuge (5000 rpm for 5 minutes) and transfer the supernatant to the same volumetric flask.
4. Repeat the extraction until the residue is colourless (free from pigments). Wash the mortar and pestle thoroughly with 80 % acetone and collect the clear washing in to the volumetric flask.
5. Make up the volume to 100 ml with 80 % acetone.
6. Measure the absorbance of the solution with spectrophotometer at 645, 652 and 663 nm against the solvent (80 % acetone) as blank.

Calculation:

Calculate the amount of chlorophyll present in the extract (mg chlorophyll per g fresh tissue) using the following equations:

$$\text{mg chlorophyll a g}^{-1}\text{ tissue} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll b g}^{-1}\text{ tissue} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{mg total chlorophyll g}^{-1}\text{ tissue} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

Where, A= Absorbance at specific wavelengths
V= Final volume of chlorophyll extract in 80 % acetone
W=Fresh weight of tissue extracted

References:

- Sadasivam, S. and Manickam, A. 1992. In: *Biochemical methods for Agricultural Sciences*, Wiley Eastern Ltd., New Delhi, pp. 184-185.
- Thimmaiah, S. K. 1999. *Standard methods of biochemical analysis*. Kalyani Publishers, New Delhi, pp. 307-308.
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Estimation of Total Carotenoids in Vegetables and Fruits

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Carotenoids are tetraterpenoid (C_{40}) compounds widely distributed in plants. They function as accessory pigments in photosynthesis and as colouring matters in flowers and fruits. Some of the commonly occurring carotenoids are simple unsaturated hydrocarbons based on lycopene and their oxygenated derivatives known as xanthophylls. *β*-Carotene is the most common pigment in this group found in higher plants. A method to extract total caretenoids from fresh materials of vegetable and fruit plants in acetone and petroleum ether, respectively and their determination is described below:

Principle:

The total carotenoids are extracted and partitioned in organic solvents (acetone or methanol) on the basis of their solubility. Carotenoids that are bound as esters are hydrolyzed using aqueous 60% KOH. The amount of carotenoids present in sample is estimated spectrophotometrically at 450 nm using *β*-carotene as a standard.

Equipments:

Balance, Spectrophotometer, Rotatory evaporator or Water bath, Mortar, Pestle, Separatory funnel, Buchner funnel, Filter paper, Conical flak and Volumetric flak.

Reagents:

1. Acetone or methanol (distilled)
2. Peroxide-free petroleum ether
3. Ethanol
4. Aqueous KOH (60%)
5. Standard *β*-carotene solution

Procedure:

1. Cut the fresh sample of vegetable or fruit tissue and grind a known amount (2 g) in a mortar with 20 ml of either distilled acetone or methanol.
2. Filter the extract on a Buchner funnel through Whatman No. 42 filter paper.
3. Repeat the extraction until the tissue was free from pigments.
4. Pool the filtrates and partition thrice with equal volume of peroxide free petroleumether using a reparatory funnel (add water if necessary to produce two layers during initial ether extraction).
5. Evaporate the combined ether layers under reduced pressure at 35 ºC in a Buchii type rotatory evaporator or in hot water bath and dissolve the residue in minimum quantity of ethanol.
6. Add 60% aqueous KOH at the rate of 1 ml for every 10 ml of the ethanol extract to saponify the residue.
7. Boil the mixture with add an equal volume of water and partition the mixture twice with petroleum ether.
8. Evaporate the combined ether layers as before and dissolve the residue in minimum volume of ethanol.
9. Measure the absorbance of the solution at 450 nm with spectrophotometer and calculate the total carotenoids content (mg 100 g^{-1}) in the sample using a calibration curve prepared against a high purity *β*-carotene.

   Make suitable dilution of the acetone or methanol extract of the plant tissues and measure the absorbance at 450 and 670 nm in a spectrophotometer. If A_{450} is 10 times that of A_{670}, the total carotenoids present in the extract may be estimated from the A_{450} values using the following formula:

$$C = \frac{D \times V \times f \times 10}{2500}$$

Where,

C = Total amount of carotenoids (mg)
D = Absorbance at 450 nm in a 1.0 cm cell
V = Volume of the original extract in ml
F = Dilution factor and
2500= Average extinction coefficient of the pigments

References:

- Jenson, A. 1978. *Chlorophylls and Carotenoids*. In: Hellebust, A and J. S. Crargie (eds.) *Handbook of Phytological methods*, Cambridge university press, London, pp. 59-70.
- Mahadevan, A. and Sridhar, R. 1986. In: *Methods in Physiological Plant Pathology* (3rd edn.), Sivakami Publications, Chennai, pp. 9-11.
- Thimmaiah, S. K. 1999. *Standard methods of biochemical analysis*. Kalyani Publishers, New Delhi, pp. 304-305.

Definitions

Atomic Weight : Atomic weight of an element is the relative weight of the atom on the basis of oxygen as 16 e.g. atomic weight of sodium is 23.

Molecular Weight: The sum of the atomic weights of all the atoms in a molecule is its molecular weight.

E.g. Molecular weight of H_2SO_4 is 98, since

Hydrogen 2x1 = 2, Sulphur 1x32=32, Oxygen 4x16=64 i.e. 98

Equivalent Weight : Equivalent Weight of a substance is the number of grams of the substance required to react with, replace to react with, replace or furnish one mole of H_2O+or OH^-

The equivalent weight of an acid is the weight that contains one atomic weight of acidic hydrogen i.e., the hydrogen that reacts during neutralization of acid with base.

For example, the equivalent weight of H_2SO_4 is 49. Since H_2SO_4 contains two replaceable hydrogen, so that equivalent weight is molecular weight/2 i.e., 98/2=49

Percent solution (W/v): One percent solution of a substance contains one gram of the substance in 100ml of the solvent.

Molar Solution (M): One molar solution of a substance contains one mole Or one gram molecular weight of the substance in one litre of solution.

Eg: 1M NaOH contains 40g sodium hydroxide in one litre solution.

Normal Solution (N): One normal solution of a substance contains one equivalent of one gram equivalent weight of the substance in one litre of solution (i.e. molecular weight divided by the hydrogen equivalent of the substance) e.g. In H_2SO_4 contains 49g H_2SO_4 in one litre solution.

Buffer: A solution containing both a weak acid and its conjugate weak base whose pH changes only slightly on the addition of acid or alkali.

Molarity (M): the number of moles of solute per kg of solvent.

Preparation of Glassware

Aim: Preparation of glassware's.

Requirements:

1. Glassware:- test tubes, Beaker, conical flask, measuring cylinders.
2. Chemicals:- 70% alcohol, potassium dichromate, Conc.H_2SO_4
3. Others:- balance, Detergent ,washing tub , brush.

Procedure:-

1. Wash the glassware thoroughly with the detergent under tap water.
2. Prepare Chromic acid by dissolving 20 gm of potassium dichromate in 100 ml of Conc.H_2SO_4 and then dilute it with 100ml distilled water.
3. Now rinse the glassware's thoroughly with Chromic acid and washes under tap water till the Chromic acid is removed.
4. Again rinse the glassware with distilled water and dry them.
5. Keep them in oven at 110 ^{O}C for biochemical, microbiological and other routine work for one or two hour and then it is ready for use.
6. For microbiology and biotechnology purpose rinse the glassware's with a piece of cotton dipped in 70% alcohol dry the glassware's in air , wrap them in paper tie with thread and put them in autoclave.

Precautions

1. After rinsing the glassware's with chromic acid, it should be washed under tap water thoroughly to remove chromic acid.
2. The glassware's should be dried before wiping them with cotton dipped in 70% alcohol.
3. The glassware's must be completely dried before wrapping.

Interpretation:

The glassware's must be washed properly as to make them free of dirt and dust. Proper washing of the glassware's is an essential step to be performed before conducting any biochemical, microbiological or biotechnological experiments.

Appendix I

Conversion factors :

me.	mg/eq.wt
ppm	mg/l or µg/ml
1 ppm	2.25 kg/ha
10,000 ppm	1 per cent
1 mg/100 g	22.5 kg/ha
1 kg/ha	0.1 g /sq m
1 hectare	2.471 acres
1 acre	0.405 hectare
1 kg /ha	1.12 lbs/ acres
Mesh number	16/ mm
Normality (Liquid)	$\frac{\text{Specific gravity X Purity X 10}}{\text{Equivalent weight}}$
Normality (Solid)	$\frac{\text{Wt of salt per liter X Purity}}{\text{Equivalent weight}}$
Grams per liter	Normality X Eq. Wt.
Organic matter	Organic carbon X 1.724
Optical density	2 – log T (T= transmission)
Per cent by Wt.	$\frac{\text{Grams of solute}}{\text{100 g of solution}}$
1 Angstrom (Å)	10^{-8}cm or 10^{-10} m
10 (Å)	1 nanometer or 1 millimicrometer
^{o}C / 5	(^{o}F-32) / 9
P X 2.29	P_2O_5
P_2O_5	0.44 X P
K X 1.20	K_2O
K_2O X 0.83	K
S X 3	SO4
N X 1.12	NH_3
Protein (%)	Nitrogen (%) X 6.25
Velocity of light	3 X 10^{-10} cm / sec.

Velocity of sound 332 m / sec.

Appendix II

Percentage composition of manures and fertilizers

Material	N	P_2O_5	K_2O	Others
FYM	0.5-1.5	0.4-0.8	0.5-1.9	-
Compost (urban)	1.0-2.0	1.0	1.5	-
Green manure	0.5-0.7	0.1-0.2	0.8-1.6	-
Bone meal(steamed)	1.0-2.0	25-30	-	-
Anhydrous ammonia	82	-	-	-
Ammonium chloride	24	-	-	-
Ammonium nitrate	33	-	-	-
Ammonium sulphate	20.6	-	-	24 (S)
Ammonium phosphate	11	48	-	-
Diammonium phosphate	21	53	-	-
Calcium cynamide	20	-	-	-
Calcium nitrate	16	-	-	-
Sodium nitrate	16	-	-	-
Urea	46	-	-	-
Super phosphate	-	16	-	12 (S)
Dicalcium phosphate	-	23	-	-
Tricalcium phosphate	-	45	-	-
Rock phosphate	-	11-17	-	-
Basic slag	-	3.5-8	-	-
Muriate of potash	-	-	60	-
Sulphate of potash	-	-	48	18 (S)
Zinc sulphate	-	-	-	35 (Zn); 18 (S)
Zinc chelate	-	-	-	14 (Zn)
Copper sulphate	-	-	-	25 (Cu); 13 (S)
Ferrous sulphate	-	-	-	19 (Fe); 19 (S)
Iron chelate	-	-	-	5-9 (Fe)
Borax	-	-	-	11 (B)
Sodium tetra borate	-	-	-	14 (B)
Manganese sulphate	-	-	-	26 (Mn)
Manganese chelate	-	-	-	10-12 (Mn)
Calcium sulphate (Gypsum)	-	-	-	18 (S); 33 (Ca)

Appendix III

Ready reckoner of fertilizer schedule at varying soil test values for different crops (kg ha^{-1})

Fertilizer Name	Fertilizers Recommendations				
	Very Low	Low	Medium	High	Very High
PADDY (80:50:30)					
Urea	260	215	175	130	85
Super Phosphate	470	390	310	235	155
Muriate of Potash	75	65	50	40	25
SOYBEAN (20:80:20)					
Urea	65	55	45	35	20
Super Phosphate	750	625	500	375	250
Muriate of Potash	50	40	35	25	20
WHEAT IRRIGATED (100:50:30)					
Urea	325	270	220	165	110
Super Phosphate	470	390	310	235	155
Muriate of Potash	75	65	50	40	25
WHEAT UNIRRIGATED (30:40:20)					
Urea	80	65	30	15	-
Super Phosphate	375	310	250	125	60
Muriate of Potash	50	30	15	10	-
GRAM (30:60:30)					
Urea	100	80	65	50	35
Super Phosphate	565	470	375	280	190
Muriate of Potash	75	65	50	40	25
MOONG / URID / LENTIL / ARHAR (20:50:20)					
Urea	65	55	45	35	20
Super Phosphate	470	390	310	235	155
Muriate of Potash	50	40	35	25	25
PEA (35-75-30)					
Urea	100	80	65	50	35
Super Phosphate	700	585	470	350	235
Muriate of Potash	75	65	50	40	25
MUSTARD (60-30-20)					
Urea	195	165	130	100	65
Super Phosphate	280	235	190	140	95
Muriate of Potash	50	40	35	25	15
SUNFLOWER / SAFFLOWER (40:40:30)					
Urea	130	110	90	65	45
Super Phosphate	375	315	250	190	125
Muriate of Potash	75	65	50	40	25
MAIZE (120:80:60)					
Urea	390	325	260	195	130
Super Phosphate	750	625	500	375	250
Muriate of Potash	150	125	100	75	50
VEGETABLES (100:50:30)					
Urea	325	270	220	165	110
Super Phosphate	470	390	310	235	155
Muriate of Potash	75	60	50	40	25
JOWAR (50:35:25)					
Urea	190	160	120	90	60
Super Phosphate	270	230	180	130	90
Muriate of Potash	40	30	30	20	15

Note: In wheat, paddy, mustard and maize the 50% of urea to be applied as basal doses. Rest 50% may be applies in 2 to 3 split doses as top dressing.

Appendix IV

General recommended doses of micronutrient fertilizer

Micronutrient	Material and doses for application	
Zinc	Zinc sulphate (25-50 kg ha^{-1}) (25 kg ha^{-1} for light soils and 50 kg for heavy soils)	0.5% zinc sulphate + 0.25%lime
Iron	Ferrous sulphate (25-50 kg ha^{-1})	1-2% ferrous sulphate + half of lime
Copper	Copper sulphate (10 kg ha^{-1})	0.1% copper sulphate + 0.05% lime
Manganese	Manganese sulphate (10 kg ha^{-1})	1% manganese sulphate + 0.25% lime
Boron	Borax (10 kg ha^{-1})	0.2% borax

Appendix V

Rating of Nutrients:

Rating	Organic carbon (%)	Available N	Available P	Available K
		(kg ha^{-1})		
Very Low	< 0.3	150	< 5	< 200
Low	0.3 – 0.5	150 – 250	5 – 10	200 – 250
Medium	0.5 – 0.75	250 – 400	10 – 20	250 – 400
High	0.75 –1.0	400 – 600	20 – 40	400 – 600
Very High	> 1.0	> 600	> 40	> 600

Appendix VI

Performa for Soil Health Card

In-situ information

To be recorded while collection of sample Sample No…….

I. General Information :

Farmers Name	
Age	
Male/Female	
Education	
Address	

II. Land details :

Name of the field	Area (ha)	Survey No	Owned	Leased in/out	Irrigated/ rainfed	Soil type

III. Cropping details :

Survey number	Kharif			Rabi		
	Crop/ variety	Yield q/ha	Crop/ Variety proposed	Crop/ variety	Yield q/ha	Crop/ Variety proposed

IV. Farmers Self Assessment-Score Card :

S. No.	Parameters	Ratings	Details	Year		
				I	II	III
I	**Soil health**					
Biological activity (Deep Medium Shallow)						
1	Earthworms	Good	Many holes and casts >10 worms			
		Fair	Few holes and casts >7-5			
		Poor	Little sign of worm activity 0 - 3 worms			
2	Birds following plough	Good	Many birds follow plough / tractor during ploughing			
		Fair	Some birds			
		Poor	Very few birds /sometimes no birds at all			
Plant growth and yield						
3	Seed germination	Good	Seed come up quickly, and even emergence			
		Fair	Germination is uneven, takes one or two days more for emergence			
		Poor	Germination is very poor with high degree of unevenness			
4	Uniformity in growth	Good	Even stand in growth, uniform green colour			
		Fair	Slight variation in crop height, moderate growth and differences in colour			
		Poor	Uneven stand, stunted growth and stressed			
5	Grain yield	Good	Good yield, and quality			
		Fair	Average crop in the region,			
		Poor	Poor crop in the area and yield is very poor			

Soil Analysis Report by laboratory Incharge/Technical Person

Name of laboratory :

Date of Sampling :

S. No.	Soil properties	Kharif			Rabi		
		I	II	III	I	II	III
1.	Soil pH						
2.	EC (dsm^{-1})						
3.	Organic Carbon (%)						
4.	Available N kg/ha						
5.	Available P kg/ha						
6.	Available K kg/ha						
7.	Available S (mg kg^{-1})						
8.	Zinc (mg kg^{-1})						
9.	Iron (mg kg^{-1})						
10.	Manganese (mg kg^{-1})						
11.	Copper (mg kg^{-1})						

Irrigation facility and water quality

Date of Sampling :

Irrigated/Un-irrigated/ Source of irrigation	Open well/ borewell /tank	Kharif			Rabi		
		I	II	III	I	II	III
Average depth of ground water							
Annual average rainfall (mm)							
Normal onset of rainfall (week/month)							
Quality of irrigation water	Poor/medium/ good						

Recommendation :

Soil Testing Sample No. ……..

Details	Kharif		Rabi	
	Manure/Fertilizer		Manure/Fertilizer	
	Recommended	Applied	Recommended	Applied
FYM (tha^{-1})				
Green manure(tha^{-1})				
Nitrogen($kg\ ha^{-1}$) • Urea				
• DAP				
• Complex				
Phosphorus ($kg\ ha^{-1}$) • Super phosphate				
• DAP				
• Complex				
Potash($kg\ ha^{-1}$)				
Micronutrients($kg\ ha^{-1}$) • Zinc sulphate				
• Micronutrient mixture				
Biofertilizers • *Azosprillum*				
• *Rhizobium*				
• Phospho bacteria				

Other details :

Nearest Agriculture Department Office	
Address and Phone number	
Input dealers Address and Phone number	
Name of the bank Account Number	
Nearest KVK Address and phone number	
Soil Testing lab Address and Phone Number	


Packing Machine
पैकिंग मशीन


MICROBES RESEARCH & PRODUCTION CENTRE

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