

SS-302: ITRODUCTION TO AGRICULTURAL CHEMISTRY 3(2-1)

Mid-Course

AGRICULTURAL CHEMISTRY: INTRODUCTION AND HISTORY

Agricultural chemistry:

It is the study of both chemistry and biochemistry which are important in agricultural production, the processing of raw products into foods and beverages, and in environmental monitoring and remediation.

Agricultural chemistry is the science of chemical compositions and changes involved in the production, protection, and use of crops and livestock; includes all the life processes through which food and fiber are obtained for humans and their animals, and control of these processes to increase yields, improve quality, and reduce costs.

Chemical properties of soil:

- ✓ Electrical conductivity (EC)
- ✓ pH
- ✓ Buffering capacity of soil
- ✓ Cation exchange capacity (CEC) of soil
- ✓ Redox potential
- ✓ Oxidation reduction reaction

History of agricultural chemistry:

- ✓ Country Gentleman was an American, he founded agricultural magazine in 1831.
- ✓ In 1929, a Swedish soil chemist, Sante Mattson (1886–1980), who spent part of his career at the United States Department of Agriculture and at Rutgers University, published a series of remarkable papers in Soil Science with a lead title of “Bylaws of Soil Colloidal Behavior”.
- ✓ Crop dusting for cotton crop was first time used in Mississippi Delta in early 1922; later on it was used as regular pesticide in agriculture.

- ✓ Marshall (1935) reported the negative charge on clay minerals by using X-ray diffraction technique.
- ✓ In situ molecular scale spectroscopic studies were initiated in the mid-1980s to late 1980s.
- ✓ The first textbook on the chemistry and physics of variable charge soils appeared in 1981.
- ✓ Linus Pauling (1901–1994), an agricultural chemist was the winner of Nobel Prize for two times. Pauling made fundamental discoveries on the formation of chemical bonds in molecules and crystals and formulated Pauling's Rules that were the basis for understanding the structures of minerals such as clay minerals.
- ✓ The pesticides marketed widely after World Wars and in 1962 these chemicals were challenged for their abuse followed by movement of agrochemical regulations now called IPM.
- ✓ The emergence of agricultural science: Justus Liebig and the Americans, 1840-1880 by Rossiter, Margaret W. was published in 1975, New Haven: Yale University Press.
- ✓ The application of plant growth regulators in agriculture has started in the 1930s in the USA.
- ✓ Ethylene, a naturally occurring substance, is one of the first plant growth regulators being discovered and used successfully for enhancing flower production in pineapple.
- ✓ The natural auxin, indole-3-acetic acid, was identified in 1930s and introduced in agriculture in 1944.

History of Agrochemicals in Pakistan

- ✓ Chemical pesticides were first used in Pakistan in the 1950s to combat locust attacks.
- ✓ After the partition of the sub-continent, Government of Pakistan initiated the Rapid Soil Fertility Survey and Population of Fertilizers project in 1958 in collaboration with the Food and Agricultural organization (FAO).
- ✓ Ayub Khan's green revolution (1958) emphasized on the fertilizer sector in the Public Sector.
- ✓ The NFC set up Pakistan's first fertilizer plant in 1958.

- ✓ The first private sector plant was set up by Exxon at Dharki in 1965 at a cost of \$43 million. This was followed by Fauji Fertilizer in 1977 and Dawood Hercules later on.

SCOPE AND CONTRIBUTION OF AGRICULTURAL CHEMISTRY

- To evaluate the nutrient supplying capacity of soil for better production of quality food grains, cereals, poultry and cattle feed.
- To assess the quality of raw products for better use of raw food in to food and beverages.
- To access the quality of irrigation water, fertilizer, and soil for effective use of available agriculture resources (soil, water and fertilizer) to grow crop plants.
- To monitor the environment quality and remedial measures for sustainable agriculture and environment.
- To understand the causes and effects of biochemical reactions related to plant and animal growth.
- To develop opportunities for controlling those reactions, and develop chemical products that will provide the desired assistance or control.
- We can say agricultural chemistry is a thread that ties together genetic, physiology, microbiology, entomology and other sciences those contribute to agricultural production, protection and processing.

Soil pH

It is defined as the -ve log of the hydrogen ion activity of a soil.

$$\text{pH} = -\log (\text{H}^+)$$

Where (H^+) represents the hydrogen ion activity in mol L^{-1} . The pH scale is the logarithm to the base 10 of the reciprocal of the hydrogen ion activity. As the pH of a solution goes from 7 to 6, the hydrogen concentration increases 10 times and OH ions decrease by 10 times. The pH scale extends from 1 to 14, with pH 7 as being the neutral point. Soils with pH less than 7 are acidic and those with a pH above 7 are alkaline or basic. This means that at pH 7, hydrogen and hydroxyl ion concentrations are equal at 10^{-7} moles per liter (e.g water).

Importance of soil pH:

1. It is major factor in determining which trees, shrubs or grasses will dominate the land under natural conditions.
2. pH influences the processes involved in the formation and development of soils.
3. Most minerals are soluble in acid soils than in alkaline soils thus releasing ions toxic to plants e.g Al.
4. It affects the availability of nutrients to the plants. Alkaline pH reduces the solubility of all the micronutrients (particularly Fe, Zn, Cu & Mn) except Mo and Cl. Within a pH range of 6.5 – 7.5, most of the essential nutrients (especially phosphate) are available to plants.
5. The soil pH also affects plant growth by influencing the activity of beneficial soil microbes. Most N-fixing bacteria are not very active in strongly acidic soils. Bacteria that decompose soil organic matter and thus release nitrogen and other nutrients for plant use are depressed by strong acidity. Fungi usually tolerate acidity better than do other microbes.
6. Plant growth is also affected at high pH due to an excess of sodium ions both in soil exchange complex and solution, which actually deteriorate soil's physical conditions for plant growth. Moreover, nutrient imbalance and sodium toxicity may also decrease plant growth.

SOIL COLLOIDS AND CLAYS

Soil Colloid (Soil Colloidal Particles)

A soil colloid may be organic or inorganic in nature which has following characteristics:

- i. Very small particle size ($\leq 1 \mu\text{m}$)
- ii. Large surface area per unit mass
- iii. Too small to be seen with ordinary light microscope
- iv. And has positive or negative charge on its surface

Types of soil colloids

There are two types of soil colloids;

1. Inorganic colloid (Clay)
2. Organic colloid (Humus)

Inorganic/mineral colloids

Inorganic colloid can be further classified into following classes

- a) Layer silicate clays (Phyllosilicates)
- b) Iron and aluminum oxide and hydroxide clays
- c) Allophanes and associated amorphous clays

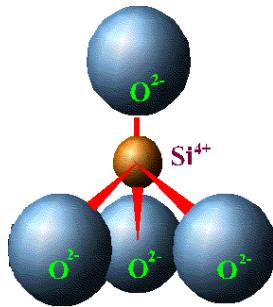
Only the layer silicate will be included in this course.

a) Layer silicate clays (Phyllosilicates; Greek phyllon, leaf)

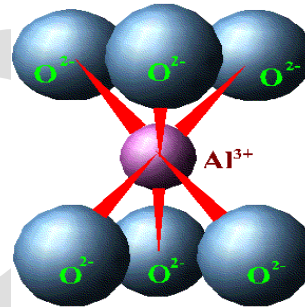
These have crystalline, layer like structures with an orderly internal arrangement. Each particle is made up of a series of layers much like the pages of a book. The layers are made up of sheets of silicon, aluminum, magnesium, and/or iron atoms surrounded by oxygen and hydroxyl groups. These are made up of two types of sheets.

1. Tetrahedral Sheets: Tetrahedral sheets consist of tetrahedron (Basic Structural Unit). Tetrahedron consists of Si^{4+} surrounded by 4 equidistant oxygen atoms forming a structure having four sides. Many tetrahedral units are linked together horizontally to form a tetrahedral sheet (also known as silica tetrahedral sheet).

2. Octahedral Sheets: Octahedral sheets consist of octahedron (Basic Structural Unit). Octahedron consists of Al^{3+} surrounded by six equidistant oxygen or hydroxyls forming a structure having eight sides.

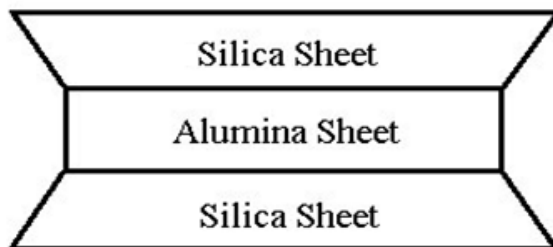


Tetrahedron

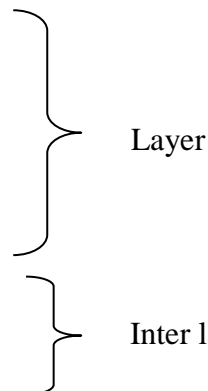


Octahedron

Many octahedral units link together horizontally to form an octahedral sheet. The tetrahedral and octahedral sheets are the fundamental structural units of silicate clays. These sheets are bound together within the crystals by shared oxygen atoms into different layers. The specific nature and combination of sheets in these layers vary from one type of clay to another and largely control the physical and chemical properties of each clay.



Adsorbed cations and water



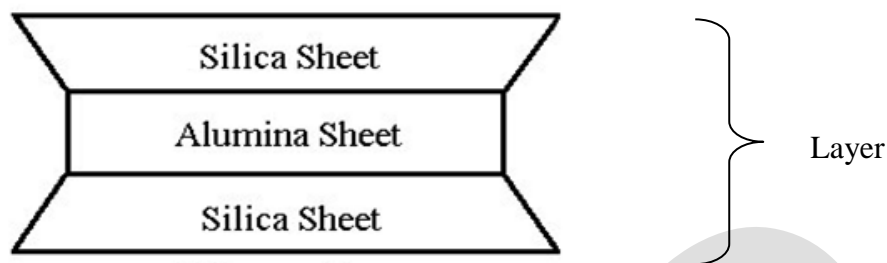


Fig. The basic molecular and structural components of layer silicate clays.

Types of silicate clays

On the basis of number and arrangement of tetrahedral and octahedral sheets, silicate clays are classified into three different groups.

Two of these groups will be discussed here:

- a) 1:1 type silicate clay: A single silica sheet (Tetrahedral sheet) attached to a single alumina sheet (Octahedral sheet). e.g. Kaolinite
- b) 2:1 type silicate clay: An alumina sheet (Octahedral Sheet) is sandwiched between two silica sheets (Tetrahedral Sheets).

e.g. Vermiculite, smectite and mica

Structures of silicate clays follow the sequence as under:

Tetrahedron	→	Tetrahedral sheet	} Both combine to form layer
Octahedron	→	Octahedral sheet	

Organic soil colloids (Humus)

Like clay, microscopic humus particles carry negative charges to which cations are attracted. The humus is composed of carbon, hydrogen and oxygen rather than aluminum silicon and oxygen like the silicate clays. The organic colloidal particles vary in size but they may be at least as small as the silicate clay particles. The humus colloids are amorphous and are not stable like inorganic soil colloids.

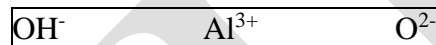
Sources of negative charges

There are two sources of negative charges on soil colloids.

- a. The charge produced due to isomorphous substitution and broken edges of clays. This type of charge is known as **permanent charge**.
- b. Hydroxyl ions or other functional groups are present on the surfaces of colloidal particles (clay and humus). These functional groups can release or accept hydrogen ions (H^+) and thus produce charge. This type of charge is known as **pH-dependent or variable charge**.

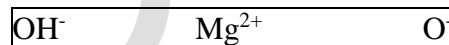
a. Permanent Charge:

Permanent charge is originated due to isomorphous substitution. **Isomorphous substitution** is the replacement of one cation by the other cation having similar size but different charge in structure of silicate clay mineral. A net negative charge arises when a lower-charged cation (lower valent) replaces a higher-charged cation (higher valent). For example Mg^{2+} , Fe^{2+} , Zn^{2+} replace higher charged cation (e.g., Al^{3+}) during the crystallization of the silicate clay. In some clays, aluminum ion substitutes for a silicon ion in the outer layers, while magnesium may substitute for aluminum ion in other clays. This decreases the positive charges which results in an excess of negative charges.



No charge

(No substitution)



one excessive negative charge

(Mg^{2+} substituted for Al^{3+})

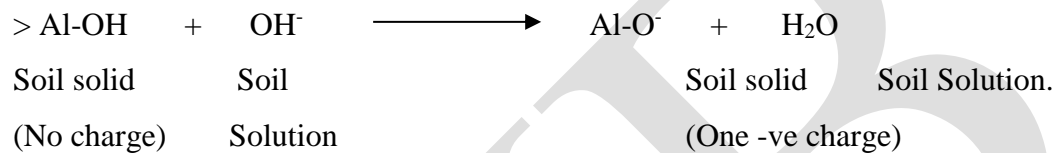
These types of charge may occur in 1:1 type clay minerals but are the main source of charge in the 2:1 type minerals. These are also called permanent charges or constant charges.

The permanent charge may also be produced due to broken edge of the clay structure.

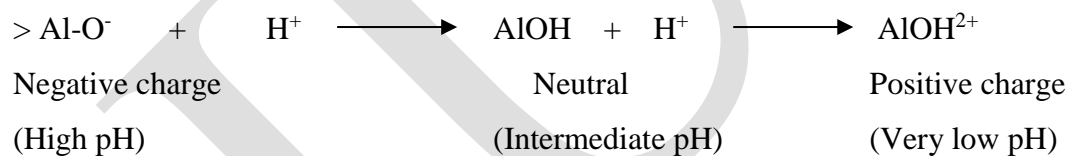
b. Variable charge:

Depending on the activity of H^+ in the soil solution, either hydrogen ion (H^+) is added to the structure (protonation) or released from the structure (deprotonation). Addition or loss of H^+ from silicate structure results in the decrease or increase of charge on the structure. So this

charge is called a pH- dependent charge or a variable charge. It is the main source of charge in 1:1 type layer silicates and humus. At the exposed crystal edges and flat external surfaces of minerals, the covalently bonded hydrogen's of hydroxyls dissociate at pH level of more than 7 leaving negative charges carried by oxygen. The loosely bonded hydrogen is readily exchangeable.



In some cases, the inorganic soil colloid may be responsible for -ve charge (high pH), no charge (intermediate pH) or +ve charge (low pH) due to fluctuation in pH as demonstrated below.



So the primary source of variable charge is due to loss or gain of H^+ from functional groups on the surfaces of soil solids. These functional groups include hydroxyl (-OH) in inorganic colloid (clay) and carboxyl (-COOH), phenolic (-C₆H₄OH) and amine -(NH₂) in organic colloid (humus).

The humus colloid has functional groups containing covalently bonded hydrogen which dissociates with the increase in pH to produce negative charges on the humus colloid.

ION EXCHANGE

It is interchange of ions between colloids and soil solution (water having dissolved salts in the soil) and/or between the solid phases if these are in a close contact. If the process is between cations, it is called cation exchange and for anions it is termed as anion exchange. These are reversible reactions.

Cation Exchange

Cations are positively charged ions. Soil colloids have negative charges on their surfaces. Cations are adsorbed at these negatively charged sites. The adsorbed cations can be exchanged by other cations present in soil solution. This exchange of one positive ion by another is called cation exchange. For example, when an NH_4^+ containing fertilizer is added to a soil, many of the numerous NH_4^+ ions replace the other cations that are already adsorbed to the exchange sites. Cation exchange takes place on the surfaces of clay and humus colloids as well as on the surfaces of plant roots. The cations mostly present on the cation exchange sites of the soil colloids are Ca^{2+} , Mg^{2+} , H^+ , Na^+ , K^+ , and Al^{3+} .

Cation Exchange Capacity (CEC)

It is defined as the total exchangeable cations that a soil can hold at a specific pH.

It is usually reported in centimoles of charge per kilogram dry soil (cmol (+) kg^{-1} soil).

Factors affecting CEC

1. pH

The CEC of a soil changes with a change in pH. As the pH rises, pH dependent CEC also increases. Most CEC from humus is pH dependent and up to 10-40% of the soil CEC may be from pH dependent charges.

2. Amount of clay

The CEC of soil generally increases with the increase in clay contents. For every 1% clay, a CEC value of 0.5 can be established. For example a soil having 40% clay, the CEC contributed by the clay will be $(40 \times 0.5) \text{ cmol (+) kg}^{-1}$ soil.

3. Type of clay

The soils having 2:1 type of clays (smectite) have a higher CEC than those having 1:1 type (kaolinitic) clays because smectite clays have more isomorphic substitution than do the kaolinite clays.

4. Amount of organic matter

Soils having a large amount of organic matter have a higher CEC than those having the same amount and types of clay but less organic matter. For every 1% well decomposed organic matter (humus) in soil, a CEC value of 2 can be established. For example, a soil having 2% organic matter will have $(2 \times 2) 4 \text{ cmol (+) Kg}^{-1}$. For a soil having 40% clay and 2% organic matter, the CEC of that soil will be approximately equal to $24 \text{ cmol (+) kg}^{-1} \text{ soil}$.

Clay (40%)	40×0.5	$= 20 \text{ cmol (+)kg}^{-1} \text{ soil}$
Organic matter (2%)	2×2	$= 4 \text{ cmol (+) kg}^{-1} \text{ soil}$
Total		$= 24 \text{ cmol (+)kg}^{-1} \text{ soil}$

Significance of cation exchange capacity of soil

1. Cation exchange is important because the cations held on the exchange complex are available to plants. These cations supplement the soil solution.
2. By cation exchange, hydrogen ions from the root surface and microorganisms replace nutrient cations from the soil exchange complex. The replaced nutrient cations can go to the soil solution.
3. Soils with high CEC have less leaching losses of nutrient cations, than the soils with low CEC.
4. It is important reaction in causing and correcting soil acidity and alkalinity. In sodic soils, the application of gypsum, a source of Ca^{2+} , to replace Na^{+} from the clay complex is a good example of the cation exchange in improving physical properties of the soils.

5. Cation exchange is also important as a mechanism in the purification and alteration of the percolating water. Cation exchange sites adsorb many metals like Cd^{2+} , Ni^{2+} , Pb^{2+} and Zn^{2+} that might be present in the waste waters.

ORGANIC MATTER

Soil organic matter is composed of decomposing residues (plants and animals), by-products formed by decomposition, microorganisms and materials resistant to further decomposition. Generally, a mineral soil contains 2-5 % organic matter. Organic matter plays a variety of roles in nutrient, water and biological cycles in soil system. Pakistani soils contain < 1 % organic matter.

Source of Organic Matter

Common organic waste materials which could be used to increase soil organic matter include animal manures, crop residues, composts, green manure, sewage sludge, food processing wastes, industrial organic wastes, wood manufacturing wastes and municipal wastes.

Significance of Organic Matter

1. Organic matter is a source of nutrients like N, P and S for plants.
2. Organic matter affects both the physical and chemical properties of the soil and its overall health.
3. It increases water infiltration in soil.
4. It increases water holding capacity. Organic matter can hold 20 times moisture of its weight.
5. Organic matter may bind pesticides making them less active, and reduces the risk of pollution.
6. It increases cation exchange capacity and thus nutrient holding capacity of soil.
7. Organic matter enhances the biological activity of soil, therefore crop health and vigour is improved.
8. It chelates (binds) nutrients and thus improves their availability.
9. It is a source of energy for soil microorganisms.
10. It buffers changes in soil pH.
11. It improves physical condition of soil.
12. It gives dark colour to soil and so influences soil temperature.

13. Organic matter is a source of growth-promoting substances for plants.

Factors Affecting Organic Matter in Soil

The amount of organic matter in soil is the result of two processes: the addition of organic matter (roots, surface residue, manure, etc.) and the loss of organic matter through decomposition. The following five factors affect both additions and losses.

1- Management. Practices that increase plant growth on a field (cover crops, irrigation, etc.) will increase the amount of roots and residue added to the soil each year. On the other hand, intensive tillage decreases organic matter by speeding up decomposition.

2- Soil texture. Fine-textured soils can hold much more organic matter than sandy soils. Decomposition occurs faster in well-aerated sandy soils. A sandy loam rarely holds more than 2 % organic matter.

3- Climate. High temperatures speed up the degradation of organic matter. In areas of high precipitation (or irrigation) there is more plant growth and therefore more roots and residues are added to the soil as source of organic matter.

4- Vegetation. In grasslands, organic matter is added to the soil each year from grass roots that extend deep into the soil. In forests, the organic matter comes from leaves that are dropped on the surface of the soil.

Soil organic matter can be improved by various practices such as addition of farmyard manure, management of crop residues and green manuring.

Green Manuring A growing crop that is plowed under and mixed with the soil to enrich the organic matter, is called green manure crop. The practice of plowing under or soil incorporation of such green manure crops is called green manuring. Green manures are mostly leguminous crops which are high in N contents. Generally following crops are suitable for green manuring: **Jantar, Guara, Cow peas, Alfalfa, Sun hemp and Clover etc.**

Composting is a controlled biological process which converts organic constituents, usually wastes, into humus like material suitable for use as a soil amendment or organic fertilizer.

SOIL WATER

It is the water which is present in soil pores or in the form of soil solution.

The availability of water in soil is essential for plant growth. It is also essential to microorganisms that grow in soil and decompose organic matter. It is important in the weathering process which involves the breakdown of rocks and mineral to form soil and release plant nutrients. Water is the solvent that together with the dissolved nutrients make up the solution from which plants absorb nutrients (mainly through the roots). Soil water can provide control over both soil air and soil temperature (other two factors essential for plant growth). Water is generally held in the soil by micro pores. The force of gravity causes water to move downward through the soil, particularly in the larger pores.

Importance of Soil Water

Soil water is important for three special reasons:

1. The presence of water is essential for the all life on Earth, including the lives of plants and organisms in the soil.
2. Water is a necessary for the weathering of soil. Areas with high rainfall typically have highly weathered soils. Since soils vary in their degree of weathering, it is expected that soils have been affected by different amounts of water.
3. Soil water is the medium from which all plant nutrients are assimilated by plants. Soil water, sometimes referred to as the soil solution, contains dissolved organic and inorganic substances and transports dissolved nutrients, such as nitrogen, phosphorus, potassium, and calcium, to the plant roots for absorption.

Soil Water Potential

The difference between the free energy of soil water and that of pure, free water in a standard reference state is known as soil water potential. The soil water potential can be expressed in bars, atmospheres or kilopascals (Kpa).

Water Retention Forces

Due to the uneven distribution of charge, the water molecule is polar in nature. The hydrogen atoms are attached to oxygen in a nearly V-shaped arrangement at an angle of 105° . The hydrogen atom side of the water molecule tends to be electropositive and opposite side electronegative, even though the molecule is electrically neutral. Due to this, there is attraction of water molecule for solid surfaces (called adhesion or adsorption) and also attraction of one water molecule to another (called cohesion or cohesive bonding). Strong combined adhesion and cohesion forces make possible for the soil to attract and hold water, and control its movement and use.

Soil Water Classification

The most useful classification of water content in soil is the biological classification as it relates water to plant growth. There is a definite relationship between water retention and its use by plants. These water contents are classified as gravitational (drainage) water, field capacity, permanent wilting percentage and plant-available water.

a. Gravitational Water

Water present in excess of field capacity or water held at a potential greater than -33 kPa (-10 to -33 kPa and upward depending upon soil texture) is called gravitational water. It is available as it moves through the plant roots if adequate aeration is maintained. It is of limited use to plants as it is present in the soil only for short periods of time. It can affect plant growth due to poor aeration.

b. Field Capacity

The content of water remaining in a soil two to three days after having been saturated with water and after the free water (gravitational water) has been allowed to drain away is called field capacity. It is the percentage of soil water that is held in the soil at a water potential less than -10 to -33 kPa (depending on soil type) and is a measure of the greatest amount of water that a soil, can store (or hold).

c. Permanent wilting percentage

It is the largest water content of soil at which plants growing in that soil will wilt and not recover when placed in a humid chamber. It is estimated at about -1500 kPa water potential or less (more negative). Water is held so strongly that plants are not able to absorb it fast enough for their, needs.

d. Plant available water

Plant-available water is defined as the weight percentage of total soil water held with a water potential between -10 to -33 and -1500 kPa and is said to be usable by plants. It is estimated by subtracting the percentage of water held at the permanent wilting point from the percentage held at field capacity.

Gravitational water content refers to the amount of water held by the soil between saturation and field capacity.

Water holding capacity refers to the amount of water held between field capacity and wilting point.

Volumetric water content: The volumetric water content measured is the total amount of water held in a given soil volume at a given time. It includes all water that may be present including gravitational, available and unavailable water.

Methods for Measurement of Soil Water

The most direct method for measuring soil water is the gravimetric method. Soil water can be indirectly measured by calibration of instrument against known water contents. These are as follows.

- a) Tensiometer
- b) Resistance Blocks
- c) Neutron probe
- d) Soil psychrometers
- e) Gamma ray absorption

SOIL ELECTRICAL CONDUCTIVITY (EC)

Generally, electrical conductivity is ability of electric current to flow through a material.

Electrical conductivity is the ability of a material to conduct (transmit) an electrical current and it is commonly expressed in units dS m^{-1} . Electrical conductivity of soil extract is measured with the use of electrical conductivity meter in laboratory. Soil electrical conductivity is an indirect measurement that correlates very well with several soil physical and chemical properties. Actually soil EC is a measure of the amount of salts in soil.

Units of EC

The standard units of EC are dS m^{-1}

$$\mu\text{S cm}^{-1} = \text{dS m}^{-1} \times 1000$$

Criteria for the Fitness of Soil

- ✓ If $\text{ECe} \leq 4000 \mu\text{S cm}^{-1}$ -----Normal Soil
- ✓ If $\text{ECe} = 4000$ to $8000 \mu\text{S cm}^{-1}$ ----- Saline Soil (Only salt tolerance species)
- ✓ If $\text{ECe} > 8000 \mu\text{S cm}^{-1}$ Extremely Saline Soil (Only kallar grass etc)

Importance of Electrical conductivity:

1. It is an important indicator of soil health.
2. Soil electrical conductivity is a measure of the amount of salts in soil (salinity of soil).
3. It affects crop yields, crop suitability, plant nutrient availability, and activity of soil microorganism which influence key soil processes including the emission of greenhouse gases such as nitrogen oxides, methane, and carbon dioxide.
4. Excess salts hinder plant growth by affecting the soil-water balance.
5. Soils containing excess salts occur naturally in arid and semiarid climates. Salt levels can increase as a result of cropping, irrigation, and land management.
6. Although EC does not provide a direct measurement of specific ions or salt compounds, it has been correlated to concentrations of nitrates, potassium, sodium, chloride, sulfate, and ammonia.

Buffering capacity of soil

Buffering capacity is the ability of soil to resist changes in pH. Soils with a high buffering capacity require a great deal of amendment to alter pH. This is good if the soil already has a desirable pH, but it can be a problem if the soil needs pH modification. Normally, soils high in clay or organic matter (those that have high CECs) have high buffering capacities. Calcareous soils often have high buffering capacities because lime effectively neutralizes acid—a great deal of acidification may be necessary to eliminate the lime before you can realize a significant drop in pH. Conversely, in lime-free soils, acid treatment can drop pH significantly. Soils also can resist upward changes in pH, depending on their composition. Because buffering capacity determines how much amendment it will take to change pH, this is an important characteristic. Soil labs determine buffering capacity and adjust their recommendations according to the buffer pH.

Final Course

ESSENTIAL PLANT NUTRIENTS

Plants need food for their growth and development like other living things. Humans and animals depend on plants for their food but plants can produce their food from natural raw materials. Nineteen elements have been found to be indispensable for plant growth, development and reproduction. These essential elements are referred to as essential nutrients.

Criteria of essentiality

An element should meet following three criteria to be termed as an essential nutrient:

1. Plant is unable to complete vegetative or reproductive stage of its life without that element.
2. The need of such a nutrient is specific and its deficiency symptoms can be corrected by supplying only the same nutrient.
3. The nutrient plays a direct role in plants active (metabolic) processes and meets its nutritional needs.

Essential nutrients can be distinguished into macro and micro nutrients depending upon their requirements.

Macro nutrients (major nutrients)

Macro nutrients are required in relatively larger quantity by plants. Their concentration in plants is usually $> 500 \text{ mg kg}^{-1}$.

i.e. C, H, O, N, P, K, Ca, Mg, S

Macro nutrients are further categorized into primary and secondary nutrients

From a management perspective, the primary nutrients are N, P, and K, because they are most often limiting from a crop production standpoint. All of the other essential

macronutrient elements (Ca, Mg and S) are secondary nutrients because they are rarely limiting, and seldom added to soils as fertilizers

Micro nutrients (minor nutrients/ trace elements)

Micro nutrients required in relatively smaller quantity by plants. Their concentration in plants is usually $<100 \text{ mg kg}^{-1}$. i.e. Zn, Cu, B, Fe, Mn, Cl, Mo,

Beneficial elements

Sodium, Cobalt, Nickel, Silicon, Vanadium, Selenium

Sources

C, H, O, are available to plants naturally from air and water. For other nutrients many sources can be used but commonly used are as,

N= Urea $(\text{NH}_2)_2\text{CO}$, Ammonium Sulfate $(\text{NH}_4)_2\text{SO}_4$, Calcium ammonium nitrate (CAN), Di-ammonium phosphate (DAP), Nitrophos (NP)

P= Single super phosphate (SSP), Triple super phosphate (TSP), Di-ammonium phosphate (DAP), Mono-ammonium phosphate (MAP) and Nitrophos (NP)

K= Sulfate of potash (SOP) and Murate of potash (MOP)

Ca= Calcium sulfate $(\text{CaSO}_4 \cdot 2\text{H}_2\text{O})$

Mg= Magnesium sulfate (Mg SO_4)

S= Elemental sulfur (S)

Zn= Zinc sulfate (ZnSO_4)

Cu= Copper sulfate (CuSO_4)

Fe= Iron sulfate (FeSO_4)

Mn= Manganese sulfate (MnSO_4)

Cl= Sodium chloride (NaCl), Potassium chloride (KCl)

Mo= Ammonium molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 2\text{H}_2\text{O}$

B= Boric acid (H_3BO_3)

In addition to these mineral fertilizers some organic sources/ biofertilizers are also used to supply these nutrients

Available forms:

Nutrients	Cations	Anions
Macronutrients		
Nitrogen	NH_4^+	NO_3^-
Phosphorus		HPO_4^{2-} and H_2PO_4^-
Potassium	K^+	
Magnesium	Mg^{2+}	
Calcium	Ca^{2+}	
Sulfur		SO_4^{2-}
Micronutrients		

Copper	Cu^{2+}	
Chlorine		Cl^-
Iron	Fe^{2+}	
Boron		H_3BO_3
Manganese	Mn^{2+}	
Zinc	Zn^{2+}	
Molybdenum	MoO_4^{2-}	

ROLE OF MACRONUTRIENTS FOR PLANT GROWTH

NITROGEN (N)

Mobility in Plants: Mobile

Mobility in Soil: Mobile as NO_3^- , immobile as NH_4^+

Plant Uptake

- NO_3^-
- NH_4^+

N Fertilizers

- N= Urea $(\text{NH}_2)_2\text{CO}$
- Ammonium Sulfate $(\text{NH}_4)_2\text{SO}_4$
- Calcium ammonium nitrate (CAN)
- Di-ammonium phosphate (DAP)
- Nitrophos (NP)

Function

- Nitrogen is a part of all living cells and is a necessary part of all proteins, enzymes and metabolic processes involved in the synthesis and transfer of energy.
- Nitrogen is a part of chlorophyll, the green pigment of the plant that is responsible for photosynthesis.
- Helps plants with rapid growth, increasing seed and fruit production and improving the quality of leaf and forage crops.

Deficiency Symptoms

- Reduced growth
- Yellowing of old leaves

PHOSPHORUS (P)

Mobility in Plants: Somewhat mobile

Mobility in Soil: Immobile

Plant uptake:

- HPO_4^{2-}

- H_2PO_4^-

P Fertilizers

- P= Single super phosphate (SSP)
- Triple super phosphate (TSP)
- Di-ammonium phosphate (DAP)
- Mono-ammonium phosphate (MAP)
- Nitrophos (NP)

Functions

- The most important function of P in plants is energy storage and transfer. Phosphorus is an integral part of Adenosine triphosphate (ATP) and Adenosine diphosphate ADP.
- It is involved in the formation of all oils, sugars, starches, etc.
- Adequate supply of P is important for development of reproductive parts of plant.
- It improves blooming and root growth.
- It hastens crop maturity.

Deficiency Symptoms

- P is mobile in plant tissues (Deficiency occurs in older leaves)
- Dark, purplish color on older leaves

POTASSIUM (K)

Mobility in Plants: Very mobile

Mobility in Soil: Somewhat mobile

Plant uptake

- K^+

K Fertilizers

- Sulfate of potash (SOP)

- Murate of potash (MOP)

Function

- Potassium, unlike N and P forms no compounds in the plant.
- It is required for the activation of more than 80 enzymes. This is the most important function of K.
- It is involved in stomatal regulation. It helps in the maintenance of plant turgor.
- Plants require K for better produce quality.

Deficiency Symptoms

- Leaf margin necrosis and browning
- Older leaves are more affected

CALCIUM (Ca)

Mobility in Plants: Immobile

Mobility in Soil: Somewhat mobile

Plant uptake

- Ca^{+2}

Ca Fertilizers

- Calcium Fertilizers
- Calcium sulfate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$)
- Lime (CaCO_3),
- Gypsum (CaSO_4)

Functions

- Component of cell wall
- Involved in cell membrane function
- Largely present as calcium pectate

- Calcium pectate is immobile in plant tissues

Deficiency Symptoms

- Deficiency symptoms in young leaves and new shoots (Ca is immobile)
- Stunted growth, leaf distortion, necrotic spots, shoot tip death

SULPHURE (S)

Mobility in Plants: Immobile

Mobility in Soil: Mobile

Plant Uptake:

- SO_4^{2-}

S Fertilizer:

- Gypsum (CaSO_4)
- Magnesium sulfate (MgSO_4)
- Ammonium sulfate $[(\text{NH}_4)_2\text{SO}_4]$
- Elemental sulfur (S)

Functions

- Component of amino acids (methionine, cysteine)
- Constituent of coenzymes and vitamins
- Responsible for pungency and flavor (onion, garlic, mustard)

Deficiency Symptoms

- Light green or yellowing on new growth (S is immobile)

MAGNESIUM (Mg)

Mobility in Plants: Somewhat mobile

Mobility in Soil: Immobile

Plant uptake:

- Mg^{+2}

Mg Fertilizer:

- MgSO_4

Function

- Core component of chlorophyll molecule
- Catalyst for certain enzyme activity

Deficiency Symptoms

- Interveinal chlorosis on mature leaves (Mg is highly mobile)

ROLE OF MICRONUTRIENTS FOR PLANT GROWTH**IRON (Fe)****Mobility in Plants:**

Immobile

Mobility in Soil:

Immobile

Plant uptake:

- Fe^{2+}
- Fe^{3+}

Fe Fertilizer:

- FeSO_4

Function

- Component of cytochromes (needed for photosynthesis)
- Essential for N fixation (nitrate reductase) and respiration
- Essential for chlorophyll production

Deficiency Symptoms

- Interveinal chlorosis on new growth
- Stunted growth and short stem

MANGANESE (Mn)

Mobility in Plants: Immobile

Mobility in Soil: mobile

Plant uptake:

- Mn^{2+}

Mn Fertilizer

- MnSO_4

Function

- Required for chlorophyll synthesis,
- O_2 evolution during photosynthesis
- Activates some enzyme systems
- Nitrogen transformation

Deficiency:

- Mottled chlorosis between main veins of new leaves
- Young leaves

BORON (B)

Mobility in Plants: Immobile

Mobility in Soil: Very mobile

Plant uptake:

- H_3BO_3

B Fertilizers:

- Borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$)
- Calcium borate ($\text{NaB}_4\text{O}_7 \cdot 4\text{H}_2\text{O}$)

Functions

- Involved in carbohydrate metabolism
- Essential for flowering, pollen germination, N metabolism
- Affects water absorption by roots

Deficiency:

- New growth distorted
- Flowering and fruit set depressed
- Roots tubers distorted
- Leaves become twisted and die

Zinc (Zn)

Mobility in Plants: Immobile

Mobility in Soil: Immobile

Plant uptake:

- Zn^{2+}

Zn Fertilizers:

- ZnSO_4

Functions

- Involved in protein synthesis, IAA synthesis
- Plant metabolism
- Helps to form growth hormone

Deficiency:

- Growth suppression
- Reduced internode lengths
- Resetting, interveinal chlorosis on young leaves

MOLYBDENUM (Mo)

Mobility in Plants: Immobile

Mobility in Soil: Somewhat mobile

Plant uptake:



Mo Fertilizers:

- Sodium Molybdate
- Ammonium Molybdate

Functions

- Vitamin synthesis
- Root-nodule bacteria also requires Mo
- Plant development

Deficiency

- Pale green, cupped young leaves
- Stunted growth
- Leaf margin burn

COPPER (Cu)

Mobility in Plants: Immobile

Mobility in Soil: Immobile

Plant uptake:

- Cu^{2+}

Fertilizers

- CuSO_4

Function

- Essential component of several enzymes of chlorophyll synthesis, carbohydrate metabolism
- Help in the use of Iron
- Helps in respiration

Deficiency

- Young leaves are small and permanently wilt
- Multiple buds at stem tip

Chlorine (Cl)

Mobility in Plants: mobile

Mobility in Soil: mobile

Plant uptake:

- Cl^-

Fertilizers

- NaCl
- KCl

Function

- Involved for photosynthetic oxygen revolution
- Acts in enzymes system

Deficiency

- Normally not existing (Only experimentally induced)

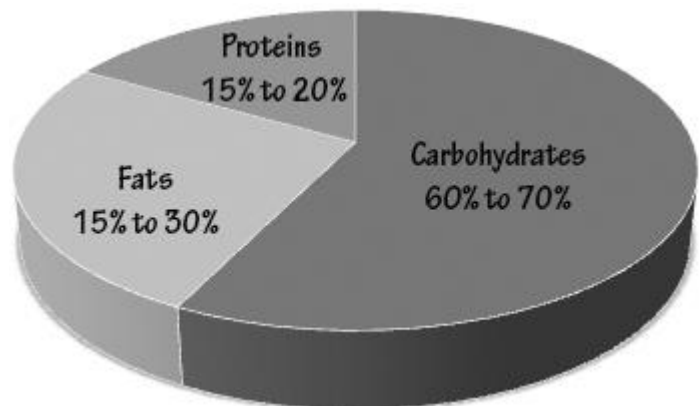
Beneficial elements

Sodium, Cobalt, Nickel, Silicon, Vanadium, Selenium

USP

CARBOHYDRATES

Carbohydrates are most abundant organic compounds found in living organisms and



are composed of carbon, hydrogen and oxygen. Carbohydrates act as the primary source to provide energy for functioning of living organisms. These are called carbohydrates because they can be considered as hydrates of carbon. Most of them have the general formula $C_x(H_2O)_y$.

Generally carbohydrates are defined as polyhydroxy aldehydes or polyhydroxy ketones or the compounds which produce such products on hydrolysis. Carbohydrates are called saccharides. Some of them have sweet taste and are called sugars.

How do you classify carbohydrates based on reactivity?

Based on the reactivity with Tollen's, Benedict's or Fehling's reagent, carbohydrates are classified as;

Reducing sugars

Carbohydrates that can reduce Tollen's, Benedict's or Fehling's reagents are called reducing sugars (sugar with free aldehyde or ketone group). All monosaccharides and most of the disaccharides are reducing sugars. Some examples are Maltose and Lactose.

Non-reducing sugars

Carbohydrates that cannot reduce Tollen's, Benedict's or Fehling's reagents are called non-reducing sugars. Sucrose is a non-reducing sugar.

LIPIDS AS BIOMOLECULES

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. Lipids are a class of compounds distinguished by their insolubility in water and solubility in nonpolar solvents.

Biological Importance

- Lipids, together with carbohydrates, proteins and nucleic acids, are one of the four major classes of biologically essential organic molecules found in all living organisms.

- Lipids are important in biological systems because they form the cell membrane, a mechanical barrier that divides a cell from the external environment.
- They are important dietary constituents not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods.
- Fat is stored in adipose tissue, where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs.
- Nonpolar lipids act as electrical insulators, allowing rapid propagation of depolarization waves along myelinated nerves.
- Combinations of lipid and protein (lipoproteins) are important cellular constituents, occurring both in the cell membrane and in the mitochondria, and serving also as the means of transporting lipids in the blood.
- Knowledge of lipid biochemistry is necessary in understanding many important biomedical areas, e.g., obesity, diabetes mellitus, atherosclerosis, and the role of various polyunsaturated fatty acids in nutrition and health.

Classification

Lipids can be divided in two major classes, nonsaponifiable lipids and saponifiable lipids.

A **nonsaponifiable lipid** cannot be broken up into smaller molecules by hydrolysis, which includes triglycerides, waxes, phospholipids, and sphingolipids. Nonsaponifiable lipids include steroids, prostaglandins, and terpenes.

A **saponifiable lipid** contains one or more ester groups allowing it to undergo hydrolysis in the presence of an acid, base, or enzyme.

On the basis of structure, the lipids can be classified into Simple lipids, complex lipids and derived lipids

Simple lipids: Esters of fatty acids with various alcohols.

(a) **Fats:** Esters of fatty acids with glycerol. Oils are fats in the liquid state.

(b) Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols.

Complex lipids: Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

- (a) **Phospholipids:** Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen containing bases and other substituents, e.g. in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.
- (b) **Glycolipids (glycosphingolipids):** Lipids containing a fatty acid, sphingosine, and carbohydrate.
- (c) **Other complex lipids:** Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.

Precursor and derived lipids: These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid soluble vitamins, and hormones. Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed neutral lipids.

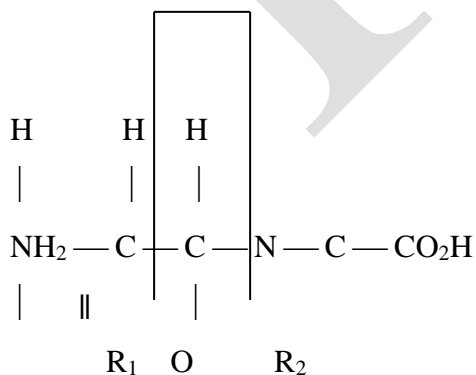
PROTEINS

Proteins are large, complex, nitrogenous biomolecules that play many critical roles in the body. They do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs.

Amino acids link together to form proteins. Proteins have vital functions including acting as enzymes, as structural components of the cell and molecular recognition. It is through the production of specific proteins that the genetic information carried in the DNA is able to control the activities of the cell.

PEPTIDE BONDS

During formation of proteins amino acids are joined together by condensation reactions to form long chains (polymers). These chains of amino acids are known as peptides and the bonds that join them are peptide bonds. The structure of a peptide bond between two amino acids is shown below:



← Peptide bond

The groups marked R_1 and R_2 are the side chains of the amino acids. This compound is an example of a dipeptide: it consists of two amino acids, but other amino acids can be added to extend the chain thus e.g. tri, tetra and penta or polypeptide chains.

PROTEIN FUNCTIONS

Proteins are polymers of amino acids linked together in straight chains to form polypeptides. They consist of between approximately 100 and 3000 amino acid residues. As the average molecular weight of an amino acid is about 110, protein molecular weight vary between about 10,000 and 300,000.

Important functions of proteins include:

- i) Their role in formation of subcellular, cellular and organic structures.
- ii) They are the constituents of the enzymes and thus are important in catalysing the biological reactions.
- iii) Many hormones are proteins. Insulin, for example, is an important proteinous hormone of the pancreas.
- iv) Most antibiotics are protein in nature. They play a key role in body defense.
- iv) The dietary proteins are important energy suppliers of the body.

CLASSIFICATION OF PROTEINS

Proteins are classified into three main groups

1. Simple proteins

These proteins on hydrolysis yield only amino acids, e.g. Albumins, globulins etc.

2. Conjugated proteins

These proteins are associated with non-proteinous groups called posthetic groups. These are further classified as bellow:

i) Nucleo-protein

Here the prosthetic group is a nucleic acid. e.g. Histones which are associated with DNA or RNA.

ii) Lipo-protein

These proteins are associated with lipid molecules such as phospholipids, cholesterol etc. and these occur in cell membrane, milk, egg yolk, etc.

iii) Phospho-proteins

These are the conjugates of proteins and phosphoric acid. e.g. Casein of milk.

iv) Glycoproteins

These contain a protein and a carbohydrate. These are found in serum, egg white etc.

v) Chromo-protein

These are composed of proteins and pigments e.g. Haemoproteins i.e. Haemoglobin and cytochromes.

vi) Metallo-protein

These proteins are coordinated to metals e.g. Carbonic anhydrase (Zn^{++})

3. Derived proteins

These are the degradation products of simple and conjugated proteins. They are further classified as:

i) Primary derived proteins:

These are simply the denaturation products of proteins by physical means (x-rays etc.) or chemical means (urea, HCl, acetone etc.).

ii) Secondary derived proteins

These are the intermediate products of hydrolysis e.g. Polypeptides, peptides etc.

Classification based on the functions of proteins

Proteins exhibit a lot of diversity in their biological functions. Therefore, these have also been classified on the basis of these functions.

1) Structural proteins

These are the structural constituents of various cells and tissues e.g. Collagen is the major extracellular protein of the connective tissue and bone. Glycoprotein form cell coat walls.

2) Storage proteins

These store certain spp. after conjugation with them e.g. albumin is the storage protein of egg.

3) Contractile proteins

These are the essential constituents of the contractile and motile systems e.g. Dynein is the contractile protein of cilia and flagella.

4) Enzymes

Enzymes catalyse biological reactions, e.g. pepsin of stomach, amylase of saliva.

The enzymes are the most important and the largest class of proteins.

5) Protective proteins

These may play an important role in the blood of vertebrates, e.g. antibodies in blood defend the body against antigen. Thrombin is involved in the clotting mechanism.

6) Transport proteins

These proteins robustly bind certain molecules and are thus capable of transporting them from one site to another via blood stream.e.g. Haemoglobin transports O_2 in the blood of some animals and myoglobin performs this function in muscle.

7) Regulatory proteins / hormones

Hormones act as chemical messengers. e.g. insulin which regulates glucose level in the blood and the vasopressin which regulates blood pressure.

8) Toxins

These are the proteins with toxic effects. Generally these are secreted by microorganisms.



ENZYMES

INTRODUCTION

Enzymes are molecules which catalyse biochemical reactions. They act on their substrates and convert them into products. Although a few examples of catalytic RNA molecules are known, these are quite unusual, and vast majority of biological catalysts are proteins.

Enzymes have a number of particularly interesting and important properties:

- ✓ They bring about changes in their substrates without being changed themselves, i.e. they are true catalysts.
- ✓ They increase the rates of reactions many thousand or even million times.
- ✓ They change the speed of reactions, but cannot make reactions occur which would not otherwise take place. Thus they change the rate but not the equilibrium constant of reactions.
- ✓ They are highly specific. This means that they will act only on substrates which have a particular structure. They convert substrates into products with almost 100% efficiency.

TYPES OF REACTIONS CATALYSED BY ENZYMES

Although thousands of different reactions are catalyzed by enzymes, they can be classified into following six basic types:

Enzyme	Type of reaction
Oxidoreductases	Catalyse oxidation and reduction reactions
Transferases	Transfer groups from one molecule to another
Hydrolases	Hydrolyse bonds by the 'addition' of water
Lyases	Catalyse the removal of groups from substrate molecules without Hydrolysis
Isomerases	Catalyse the interchange of optical or structural isomers
Ligases	Catalyse the linking together of two or more molecules.

FACTORS AFFECTING THE RATES OF ENZYME-CATALYSED REACTIONS

The rates at which enzymes convert their substrates into products depend on many factors. Their study is called enzyme kinetics and is important because enzymes determine the rates at which most vital processes occur in all living organisms.

Concentration of enzyme and substrate

Most enzyme-catalysed reactions may be described as either a zero order reaction (reaction where the rate is constant and independent of substrate concentration) or first-order reaction (reaction where the rate at any time is proportional to the existing substrate concentration).

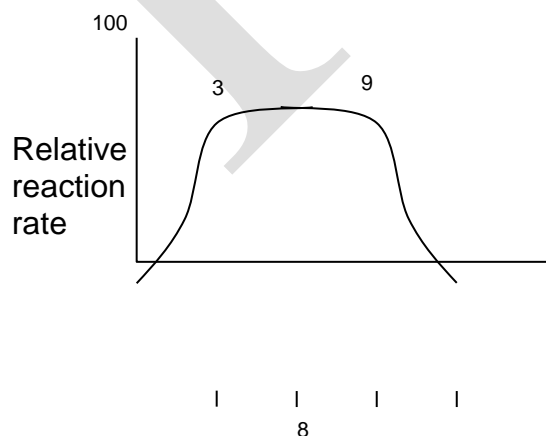
Temperature

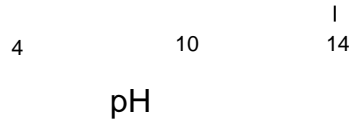
The rate of chemical reaction approximately doubles for each 10 °C rise in temp and those catalysed by enzymes behave just like any other. However, because they are proteins, enzymes are denatured by high temperature. As the temperature of an enzyme is raised, two opposing effects take place:

- i) rate is increased by the greater energy and more frequent collisions of reactant molecules;
- ii) but rate is also decreased due to enzyme denaturation.

pH

Most enzymes function best at a particular pH. Some work well over only very narrow ranges while others work over a wide range of pH.





There is an optimum pH at which the action of the enzyme is maximum. Above or below this pH the rate may diminish e.g. in the case of digestive enzymes this varies from about 1 for pepsin to 8 for pancreatic lipase. Most enzymes work most rapidly between pH 5 and 7. Changes in pH alter the number of charges on both the substrate and the enzyme, which alters the ability of groups on the enzyme and substrate to interact with one another by ionic and hydrogen bonding.

Presence of inhibitors

Inhibitors are compounds, which reduce the activity of enzymes.

Presence of coenzymes

Some enzymes require the presence of small molecules called coenzymes for activity.

Practical Course

Laboratory Safety Measures/How to work in Lab?

- As with any place of work, safety is an important consideration in soil-plant analysis laboratories, and one that is frequently overlooked.
- Safety is in the interest of the employees who work there and the organizations that operate the laboratories.
- All staff, irrespective of grade, technical skill or employment status should be briefed on all aspects of safety upon commencement of work.
- Periodic reminders of such regulations should be given to encourage familiarity with respect to regulations.

General Attitude

- Develop a positive attitude towards laboratory safety.
- Observe normal laboratory safety practices.
- Maintain a safe and clean work environment.
- Avoid working alone.

Instrument Operation

- Follow the safety precautions provided by the manufacturer when operating instruments.
- Monitor instruments while they are operating.

Accidents

- Learn what to do in case of emergencies (e.g., fire, chemical spill, etc.). Firefighting equipment must be readily accessible in the event of fire. Periodic maintenance inspections must be

conducted.

- Learn emergency First Aid. First Aid supplies are a necessity and laboratory staff should be well trained in their use. Replacement of expended supplies must take place in a timely fashion.
- Seek medical attention immediately if affected by chemicals, and use First Aid until medical aid is available.
- Access to eye-wash fountains and safety showers must not be locked. Fountains and showers should be checked periodically for proper operation.

Chemicals

- Use fume hoods when handling concentrated acids, bases or other hazardous chemicals.
- Do not pipette by mouth; always use a suction bulb.
- When diluting, always add acid to water, not water to acid.
- Many metals/salts are extremely toxic and may be fatal if swallowed. Wash hands thoroughly after handling such salts. Chemical spills should be cleaned promptly and all waste bins emptied regularly.
- All reagent bottles should be clearly labeled and must include information on any particular hazard.

Furnaces, Ovens, Hot Plates / Handling Gas

- Use forceps, tongs, or heat-resistant gloves to remove containers from hot plates, ovens or muffle furnaces.
- Cylinders of compressed gases should be secured at all times. A central gas facility is preferred.

Maintenance

- All electrical, plumbing, and instrument maintenance work should be done by qualified personnel.
- Fume hoods should be checked routinely.
- As most equipment's operate on low wattage, an Uninterruptible Power Supply (UPS) provides stable power and allows the completion of any batch measurement in the event of power outage.

1518

Continuing Education

- Display in a prominent place posters on "Laboratory Safety"
- Similarly, posters depicting First Aid after laboratories accidents should be prominently displayed.
- If the laboratory is a part of a large institution, the laboratory staff should know the Safety Officer or person responsible for safety.
- If it is a small operation, one laboratory staff member should be responsible for safety.

Contamination

The most insidious enemy in any laboratory is contamination and, therefore, its sources must be identified and eliminated. Some common sources of contamination are:

- External dusts blown from the surrounding environment;
- Internal dust resulting from cleaning operations;
- Cross-contamination derived from handling many samples at the same time (e.g., handling plant and soil samples together);
- Failure to store volatile reagents well away from the samples;
- Washing materials, particularly soap powder; and
- Smoking in the laboratory.

Eating and Drinking

- Do not eat, drink, or smoke in the laboratory. This is essential both for reasons of health and to reduce contamination. Specific areas should be designated for staff breaks.
- Do not use laboratory glassware for eating or drinking.
- Do not store food in the laboratory.

Protective Equipment

Use personnel safety equipment as follows:

- **Body Protection:**

Use laboratory coat and chemical-resistant apron.

- **Hand Protection**

Use gloves, particularly when handling concentrated acids, bases, and other hazardous chemicals.

- **Dust Mask**

Essentially needed when grinding soil, plant samples, etc.

- **Eye Protection**

Use safety glasses with side shields.

Persons wearing contact lenses should always wear safety glasses in the laboratory.

Make sure that your colleagues know that you wear contact lenses.

Contact lenses should never be worn around corrosives.

- **Full-Face Shield**

Wear face shields over safety glasses in experiments involving corrosive chemicals.

- **Foot Protection**

Proper footwear should be used; sandals should not be worn in the laboratory

Waste Disposal

- Liquid wastes should be poured carefully down a sink with sufficient water to dilute and flush it away.
- Keep in mind that local ordinances often prohibit the disposal of specific substances through the public sewerage system.
- Dispose of chipped or broken glassware in specially marked containers.

Laboratory Equipment

Flame photometer

Used for the determination of K, Na, Ca, Mg, Li and Ba

Protocol: Digested soil and plant material is aspirated and unknown concentrations of different elements is determined by using standard curve.

Spectrophotometer

Used for the determination of nutrients, enzymes kinetics studies, microbial populations etc. on the basis of absorbance and transmittance properties.

Autoclave

Used to sterilize equipment and supplies by subjecting them to high pressure saturated steam (15 psi) at 121 °C for around 15–20 minutes depending on the size of the load and the contents.

Caution

Do not open the lid until pressure is 0 psi.

While opening lid stay away from the hot steam

Laminar flow hood

Provides aseptic environment for microbiological work

Prevents contamination of biological samples, or any particle sensitive materials.

Caution

Do not use when UV light is turned on

Kjeldahl apparatus

Used for determination of nitrogen in soil and plant samples

Digestion block is used for the digestion of soil and plant samples by using digestion mixture and conc. Sulphuric acid

Distillation unit is used to liberate and capture the nitrogen in samples in boric acid solution by providing alkaline conditions that liberates nitrogen as Ammonia gas

Captured nitrogen is determined by titration against 0.1N Sulphuric acid

Hot air oven

Used for dehydrating samples

Plant samples are heated up to 67 °C

Soil samples heated up to 105 °C

To dehydrate salts heated up to 105-120 °C

Muffle furnace**Used for ashing of plant material**

Determines what proportion of a sample is non-combustible and non-volatile. Temperature range up to 1000 °C

Water bath (with shaker)

Used for controlled heating without flame for unstable and flammable substances. It is used to incubate samples in water at a constant temperature over a long period of time.

Shaking incubator

Application includes cell culturing, cell aeration, microbiology, metabolism studies, bacterial culture and solubility studies.

Agitation at variable speeds to affect the growth of cell culture.

EC meter

Electrical conductivity meter (EC meter) measures the electrical conductivity in a solution.

In soil science lab it is used to measure the EC of water samples and soil extracts in order to describe the quality of water and soil

Caution

Handle with extreme care it is a fragile instrument

pH meter

pH meter measures the pH of any solution.

For very precise work the pH meter should be calibrated before each measurement by using prescribed buffer solutions.

Caution

Handle with extreme care it is a fragile instrument

Magnetic stirrer

Used to stir the solutions to dissolve solutes. It employs a rotating magnetic field to cause a stir bar immersed in a liquid to spin very quickly, thus stirring it.

Mechanical Shaker

Used to agitate the solutions to dissolve solutes or suspend the particles in the solution to prevent their settlement in bottom of the container.

Moisture analyser

Used to determine the moisture contents in samples

It provides the measurement moisture contents automatically

Identification of Manure and Fertilizers

Manure:

Manure are bulky in nature having low analytical value and have no definite composition.

Most of these are obtained from animals or plants waste product.

Types of manure:

1. Farm yard manure
2. Green manure
3. Poultry manure
4. Biogas and biogas slurry
5. SFC (sugar felter cane) it is very precious in nutrients.
6. City and municipal wastes.

Fertilizers:

Mined or manufactured material containing one or more than one potentially enrich essential plant nutrients in available form.

Fertilizer refers to a soil amendment that guarantees the minimum percentages of nutrients (at least the minimum percentage of nitrogen, phosphate, and potash). **Or**

Fertilizers are compounds given to plants to promote growth; they are usually applied either through the soil, for uptake by plant roots, or by foliar feeding, for uptake through leaves.

Difference between manure and fertilizer

Manure

1. Manure derived from French word “manoeuvrer” meaning to work soil.
2. Organic in nature and naturally occurring.
3. Manures are slow acting and have low analytical value.
4. Manures have no definite chemical composition and obtained from plants, animals or human resources.
5. Manures improves physical properties of soil and are bulky in nature.
6. Manures supply almost all major essential plant nutrients.
7. Examples:

Farm yard manure, Green manure

Fertilizer

1. Fertilizers derived from word “fertile” meaning soil.
2. Inorganic in nature and are synthetic.
3. Fertilizers are quick active and have high analytical value.
4. Fertilizers have definite chemical composition $(\text{NH}_4)\text{Co}$ and are manufactured commercially.
5. Fertilizers do not improve in that way and are non-bulky in nature.
6. Fertilizers supply only one or few essential plant nutrients.
7. Examples:
Ammonium sulphate, DAP, Urea, single super fertilizers etc.

Types of Manure

1. Farm yard manure:

It is a mixture of cattle excreta, urine and left over chopped fodders which are heaped and decomposed anaerobically to form a well rotten black coloured manure. In general waste of cattle yard is collected in a pit and material is allowed to decomposed anaerobically.

2. Green manure:

Growing leguminous crops up to flowering and then its rotavating in the soil is called green manuring. Jantar, gauar and dahancha crops are used for green manuring. It will add lot of organic matter in soil due to symbiotic association of rhizobium and legume roots.

3. Compost manure:

Process of burying leaves and twigs of plants into soil and covering of this plant material with soil to facilitate the decomposition is called composting. The compost manure is generally used in gardens and small orchards.

Types of Fertilizers

Simple or Straight fertilizers:

These fertilizers contain only declarable contents of only one major nutrients.
e.g. urea, ammonium sulphate, MOP (muriate of potash).

Compound or Complex fertilizers:

These fertilizers as declarable amount of at least two of the major nutrients which are chemically combine during manufacture. Complex fertilizers are granular in nature.
e.g. DAP, Nitrophas.

Mixed fertilizers:

In these fertilizers, straight fertilizers are blended physically and applications of three primary nutrients (N, P, K) are provided to crops. These fertilizers contain all three primary nutrients and is also termed as NPK fertilizers. But they cannot capture market due to their big cost.

High analysis fertilizers:

These fertilizers have more than 25% of total primary nutrients in their composition.
e.g. Urea (46% nitrogen), Ammonium nitrate (26% nitrogen), Nitrophas (21% nitrogen, 21% P_2O_5), DAP (46% P_2O_5).

Low analysis fertilizers:

These fertilizers having less than 25% of primary nutrients and are called low analysis fertilizers.
e.g. Single super fertilizers (18% P_2O_5).

Fertilizers grade:

It refers to granted ingredient by manufacture on basis of lack analysis. It is the minimum quantity of nutrient present in fertilizers which is claimed by manufacturer common fertilizers are present in local market.

Complete fertilizer: It has all three primary nutrients i.e. nitrogen, phosphorous & potassium

Examples: 10-10-10, 15-30-15, 20-5-20

Incomplete fertilizer: It DOES NOT include all three primary nutrients

Examples: 20-0-0, 0-20-0, 12-0-44

Organic fertilizers

An organic fertilizer refers to a soil amendment derived from natural sources that guarantees the minimum percentages of nitrogen, phosphate, and potash. **Or**

Fertilizers which come from plant or animal matter and contains carbon compounds (composed of organic matter). They can be naturally occurring compounds such as peat or mineral deposits, or manufactured through natural processes (such as composting)

Inorganic fertilizers

Inorganic fertilizers are made of simple, inorganic chemicals or minerals). They can be manufactured through chemical processes.

Fertilizer Analysis

By law, all products sold as fertilizer require uniform labeling guaranteeing the minimum percentage of nutrients. The three-number combination (fertilizer grade or analysis) on the product identifies percentages of nitrogen (N), phosphate (P_2O_5), and potash (K_2O), respectively. For example, a 20-10-5 fertilizer contains 20% nitrogen, 10% phosphate, and 5% potash.

Fertilizer analysis expresses weight as a percent of nitrogen, phosphorus and potassium. For example 20-10-20 which means 20% N, 10% P_2O_5 and 20% K_2O .

Note: Phosphorus, P, is a primary nutrient in plant growth. The word phosphate, P_2O_5 , refers to the ionic compound containing two atoms of phosphorus with five atoms of oxygen. The phosphorus content of fertilizers is measured in percent phosphate.

The conversion between %P and % P_2O_5 is:

$$\%P = \%P_2O_5 \times 0.43$$

$$\%P_2O_5 = \%P \times 2.29$$

Note: Potassium, K, is a primary nutrient in plant growth. The word potash, K_2O , refers to the ionic compound containing two atoms of potassium with one atom of oxygen. The potassium content of fertilizers is measured in percent potash.

The conversion between %K and % K_2O is:

$$\%K = \%K_2O \times 0.83$$

$$\%K_2O = \%K \times 1.2$$

Table 1. Examples of Manufactured Fertilizers			
Product	Nitrogen %	Phosphate %	Potash %
Ammonium nitrate	34	0	0
Ammonium sulfate	21	0	0
Urea	46	0	0
Di-Ammonium Phosphate (DAP)	18	46	0
Triple Super-Phosphate (TSP)	0	50	0
Single Super Phosphate	0	18	0
Potassium Chloride	0	0	60
Potassium Nitrate	13	0	44
Potassium Sulfate	0	0	50

Physical Properties of Chemical Fertilizers

COMMERCIAL FERTILIZERS AVAILABLE IN PAKISTAN AND THEIR PHYSICAL PROPERTIES

Urea [$\text{CO}(\text{NH}_2)_2$]

This is the most concentrated solid straight nitrogen fertilizer. Its prills or granules are white in color and free flowing. Urea is readily soluble in water.. It is manufactured through Haber-Bosch process in the industries from natural gas (CH_4) and air at high temperature [$400\text{--}500^\circ\text{C}$ ($752\text{--}932^\circ\text{F}$)] and pressure [$15\text{--}25\text{ MPa}$ ($2,200\text{--}3,600\text{ psi}$) or $150\text{--}250\text{ bar}$] in the presence of metal [Fe] based catalyst.

Favorable manufacturing, handling, storage and transportation economics make urea a competitive nitrogen source. It is more advantageous than ammonium nitrate due to less stickiness and cake formation, lack of sensitivity to explosion and less corrosion to handling and application equipment. It is organic in nature that makes it different from other synthetic nitrogen fertilizers. It contains 46 percent N in amide (NH_2) form which is changed to ammonium (NH_4^+) in the soil. Because of its high water solubility, it is well suited for use in

solution fertilizers or foliar sprays. Urea, though alkaline in initial reaction, leaves behind a slightly acidic effect in the soil after nitrification.

Ammonium Sulphate [(NH₄)₂SO₄]

It is source of both nitrogen and sulfur that is less hygroscopic than ammonium nitrate. The strongly acid-forming reaction of ammonium sulphate makes it advantageous in high pH soils and for acid requiring crops.

It contains low nitrogen content (21% N) compared to other sources. It can be economical source for sulfur deficient soils.

Ammonium Nitrate

The granules are grey or light brown in color. It contains NO₃ and NH₄ form of nitrogen in equal amount [32 % N; 16% NO₃-N and 16% NH₄-N]. It is hygroscopic in nature i.e. absorbs water that results in caking during storage.

The whole fertilizer is not soluble in water but nitrogen portion of fertilizer is completely soluble making it appropriate for fertigation. It is less effective for flooded rice than urea or ammonium fertilizers. It is more prone to leaching and Denitrification than ammonium products.

Nitrophos (NP)

It is generally known as NP and is soluble in water. Its granules are brownish in color with a small depression on a side. Major part of country requirement is fulfilled by local production and Pak-ARAB fertilizer Multan contributes about 70% of total production. It contains 23% N and 23%P as P₂O₅.

NPK

The fertilizer contains all the three primary nutrients in certain concentration for specific nutrition. The fertilizer has varying degrees of color. Three primary nutrient s are mixed together physically by the process of blending in dry conditions.

Diammonium Phosphate (DAP)

DAP is manufactured by reacting wet-process H₃PO₄ with NH₃. It is available in the form of granules with various degrees of colors ranging from light grey to dark brown. It is also soluble in water and contains both ammonia and phosphorus in available form. Sometimes it is wrongly documented that DAP is not suitable for our soils due to its high pH at initial stage in soil. However, no imprecise data is available to support this statement as the final reaction of DAP in soil is acidic. Moreover, soil buffering capacity of soil is very high due to access CaCO₃ which regrets the statement of unsuitability of DAP for our soils. It contains about

18%N and 46% phosphorus as P_2O_5 . The total country demand of DAP is not fulfilled through local production.

Single Super Phosphate (SSP)

This is form of phosphatic fertilizer that is available in granular and powder form. It is grey to light brown in color. It contains large amount of $CaSO_4 \cdot 2H_2O$ (gypsum) as filler / carrier. Due to calcium availability, its application in sodic soil is more beneficial where gypsum plays its role as amendment. It is completely soluble in water. It contains 16% phosphorus as P_2O_5 and 12% sulfur.

In Pakistan, The SSP is considered as the cheapest source of phosphate content. Being acidic in nature it is supposed to be the best phosphate fertilizer for Pakistan's alkaline soils especially salt-affected soils.

Triple Super Phosphate (TSP)

It is concentrated form of phosphatic fertilizer and also available in powder, granules form. Its color is off white it is also soluble in water. The granulated form of TSP and SSP are preferred due its application and storage. It contains 46-48% phosphorus as P_2O_5 and 1.5% sulfur.

Sulphate of Potash (SOP)

It is available in white color crystalline salt. It is soluble in water. It is recommended for chloride sensitive crops in world. It is also preferred in soil with high chloride contents this fertilizer is not produced in Pakistan due to its high cost and less demand. It contains 50-52% potassium as K_2O and 18% sulfur.

Murate of potash (KCl)

This is available in crystalline salt of orange color and is completely soluble in water. it is recommended to all types of crops in Pakistan as a source of potash except chloride (Cl^-) sensitive crops like potato, tomato and tobacco. It is also preferred in soils where chloride accumulation can be problem. It contains 60% potassium as K_2O .

Zinc Sulphate ($ZnSO_4 \cdot H_2O$)

Zinc sulphate is colorless crystalline solid and is soluble in water. It is most commonly use Zn source in Pakistan. It contains 21-23% Zn.

The other sources of Zn are chelated-Zn (FeZnEDTA), ZnO and Zinc phosphate.

Experiment: Determination of Nitrogen in Fertilizer

EQUIPMENTS/APPARATUS:

Kjeldhal's distillation apparatus

Digestion tubes

Conical flasks, 500ml

Pipette, 10 ml (Bulb type)

Cylinder, 50 ml

Wash bottle

Titration unit

REAGENTS/MEDIAS:

0.1 N H₂SO₄ standardized

Sodium hydroxide; 40%

Boric acid; 4%

Bromocresol green indicator. Weigh 0.5g bromocresol green and 0.1g methyl red indicator in 100 ml of 95% ethyl alcohol.

Devarda's alloy

Digestion Mixture (9:1, K₂SO₄: CuSO₄)

METHOD:

ORGANIC FERTILIZERS (e.g Urea). Weigh accurately 0.5g sample and transfer to the digestion tube. Add 1.0g of digestion mixture Add 10-12 ml of concentrated sulfuric acid to the digestion tube. Place the tube in the digestion block. Continue heating for at 400 °C at least 2 hrs. until the contents of tube changes from black to light green or straw yellow or water white. Remove the digestion tube from digestion block and cool. Now sample is ready for distillation on distillation apparatus.

INORGANIC FERTILIZERS

Weigh accurately 0.5 g N-containing fertilizer sample in the digestion tube. Place the tube on distillation apparatus. If NO₃-Nitrogen needs to be determined, add 1.0 g Devarda's alloy in the digestion tube alongwith the fertilizer sample. Distillate on distillation unit. Nitrogen will be collected in the receiver containing 4% boric acid. Titrate against 0.1N standardized sulfuric acid from golden yellow to purple end point.

CALCULATIONS:

1 ml of 1 N H₂SO₄ = 0.0141 g N.

1ml of 0.1 N H₂SO₄ = 0.00141 g N

If

0.1 N H₂SO₄ used in back titration = C ml

Then

C ml of 0.1 N H₂SO₄ contains N = 0.00141 g N x C (where C is mL of 0.1N H₂SO₄ used)

Therefore

%N = C x 0.00141 X 100/w (A-B) = C

Where

A = ml of 0.1N H₂SO₄ used for sample titration

B = ml of 0.1 N H₂SO₄ used for blank

W = weight in gram of fertilizer sample used.

CAUTIONS / SAFETY REQUIREMENTS:

5.1 Always run blank for accuracy of results.

REFERENCE / RELATED DOCUMENTS:

Method # 970.037 AOAC. Official Methods of Analysis (1990)

PURPOSE: Determination of Phosphorus in Fertilizer**EQUIPMENTS/APPARATUS:**

100 ml volumetric flask

100 ml beaker

10 ml bulb type pipette

10 ml graduated pipette

250 ml conical flask

Bunsen burner

Wash Bottle

Whatman No.42 filter paper

Funnel with stand

Litmus paper

Weighing balance

REAGENTS/MEDIAS:

- (a) Concentrated Nitric Acid
- (b) Ammonium Molybdate Solution, 3 %
- (c) Ammonium Nitrate Solution, 50 %
- (d) Phenolphthalein indicator
- (e) Standardized 0.1 N Sulphuric Acid
- (f) Standardized 0.1 N Sodium Hydroxide

METHOD:

Weigh accurately 0.5 g phosphatic fertilizer ground, passed through 100 mesh size sieve, in 100 ml glass beaker. Add at least 50 ml distilled water and boil for 1-2 minutes. After cooling, transfer the contents to 100 ml volumetric flask and make volume up to mark with distilled water. Filter and take 10 ml solution in a 250 ml conical flask. Add 5 ml concentrated nitric acid and 15 ml 50 % ammonium nitrate solution. Heat the contents at 60 degree centigrade and then add gradually 50 ml 3% molybdate solution. Shake the conical flask during ammonium molybdate solution addition. Yellow precipitate of ammonium phosphomolybdate will form depending on the concentration of phosphorus present in the given fertilizer sample. Stay for one night. Next day filter the yellow precipitates using Whatman No. 42 and wash with ice cold distilled water till acid free. Indication of acid free precipitate is that blue litmus paper will not turn red. Now transfer the acid free precipitates along with filter paper into the same conical flask. Care should be taken that the same conical flask should also be acid free. Dissolve the precipitates completely in 0.1 N sodium hydroxide by adding 10 ml each time. Note the amount of alkali used. Now add 2-3 drops of phenolphthalein indicator to the same conical flask. Pink color will develop. Titrate against 0.1 N Sulphuric acid with continuous shaking till colorless end point. Note the volume of Sulphuric acid used.

CALCULATIONS:

$$\% \text{P}_2\text{O}_5 = \frac{0.000309 \times X - Y \times 100 \times 100}{10 \times 0.5}$$

Where

X = 0.1N NaOH used to dissolve precipitate

Y = 0.1N H₂SO₄ used for back titration.

OR

$$\%P_2O_5 = (x-y) \times 0.618$$

If 0.5 gram fertilizer sample is used

DETAIL OF CULCULATIONS

During chemical reaction out of 23 molecules of NaOH only one molecule of Na is used to form Na (NH₄) HPO₄ which contain one molecule of P.

Therefore, ammonium phosphomolybdate precipitate contains Na and P in the ratio of 1:1.

i.e., normal solution of NaOH (23gm Na/L) = 1 N solution of P (31g P / L).

$$\text{So } 31/23 = 1.3478 \text{ g P / L}$$

$$1 \text{ N NaOH} = 1.3478 \times 2.29 \text{ g P}_2\text{O}_5 / \text{L (for P to P}_2\text{O}_5 \text{ use 2.29)}$$

$$\text{-do-} = 3.0864 \text{ g P}_2\text{O}_5 / \text{litre}$$

$$\text{-do-} = 0.003086 \text{ g P}_2\text{O}_5 / \text{ml}$$

$$1 \text{ ml of } 0.1 \text{ N NaOH} = 0.0003086 \text{ g P}_2\text{O}_5 / \text{ml}$$

$$R \text{ ml of } 0.1 \text{ N NaOH} = 0.0003086 \times R \text{ g P}_2\text{O}_5 / \text{ml} \quad (R = \text{reading})$$

$$10 \text{ ml of } 0.1 \text{ sample contains} = 0.000386 \times R \quad \text{If } 10 \text{ ml aliquot is used.}$$

$$1 \text{ ml of sample contains} = 0.0003086 \times R / 10$$

$$100 \text{ ml of sample contains} = 0.0003086 \times R \times 100 / 10$$

$$0.5 \text{ gm fertilizer contain P}_2\text{O}_5 = 0.0003086 \times R \times 100 / 10$$

$$1 \text{ gm fertilizer contain P}_2\text{O}_5 = 0.0003086 \times R \times 100 / 10 \times 0.5$$

$$100 \text{ gm fertilizer contain P}_2\text{O}_5 = 0.0003086 \times R \times 100 \times 100 / 10 \times 0.5$$

So

$$\%P_2O_5 = 0.618 \times R$$

CAUTIONS / SAFETY REQUIREMENTS:

Always run a blank

REFERENCE / RELATED DOCUMENTS:

Method # 970.01 OMA. 15 Addition. Kjeldahl Plant Analysis Handbook, USA.

PURPOSE: Determination of Potassium in Fertilizer

EQUIPMENTS/APPARATUS:

Flame photometer

Electronic balance

Volumetric flask

Pipette

Wash bottle

REAGENTS/MEDIAS:

Potassium chloride: weigh 1.9103 g in 1 liter to make 1000 ppm stock solution

METHOD:

Dissolve 1.0 g ground potassium fertilizer material in 1000 ml distilled water. Shake well to dissolve. Filter the solution. Take readings of the filtrate on flame photometer using standard curve procedure. Use appropriate dilution factor in calculation.

Preparation of working standard solution:

Stock solution so prepared will be used for the preparation of working standard solutions by using formula.

$$C_1V_1 = C_2V_2$$

Where

C_1 = Concentration of stock solution in ppm

V_1 = Volume to be taken of stock solution in ml

C_2 = Concentration of K to be required in ppm

V_2 = Total volume to be required in ml

Working standard solution can be prepared of 10, 20, 30 to 100 ppm concentration.

CALCULATIONS:

$$\% K_2O = \frac{K \text{ (ppm)} \times \text{dilution Factor} \times 1.2046}{10000}$$

If 1.0 g fertilizer sample is taken then dilution factor will be 1000

So

$$\frac{K \text{ (ppm)} \times 1000 \times 1.2046}{10000}$$

Therefore

$$\% K_2O = K \text{ (ppm)} \times 0.12046$$

REFERENCE / RELATED DOCUMENTS:

Method # 971.01 AOAC. Official Methods of Analysis (1990)

ANALYTICAL METHODS

1. Determination of moisture content (AOAC,2000) Method

1. Dry the empty dish and lid in the oven at 105°C for 3 h and transfer to desiccator to cool. Weigh the empty dish and lid.
2. Weigh about 3 g of sample to the dish. Spread the sample to the uniformity.
3. Place the dish with sample in the oven. Dry for 3 h at 105°C.
4. After drying, transfer the dish with partially covered lid to the desiccator to cool. Reweigh the dish and its dried sample.

Calculation

$$\text{Moisture (\%)} = \frac{(W1 - W2)}{W1} \times 100$$

W1 = weight (g) of sample before drying W2 = weight (g) of sample after drying

2. Determination of protein content (AOAC, 2000) Reagents

- Kjeldahl catalyst: Mix 9 part of potassium sulphate (K_2SO_4) with 1 part of copper sulphate ($CuSO_4$)
- Sulfuric acid (H_2SO_4)
- 40% NaOH solution
- 0.2 N HCl solution
- 4% H_3BO_3
- Indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% bromocresol green (in 95% ethanol)

Method

1. Place sample (0.5-1.0 g) in digestion flask.
2. Add 5 g Kjeldahl catalyst and 200 ml of conc. H_2SO_4
3. Prepare a tube containing the above chemical except sample as blank. Place flasks in inclined position and heat gently until frothing ceases. Boil briskly until solution clears.
4. Cool and add 60 ml of distilled water cautiously.
5. Immediately connect flask to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5-7 drops of mix indicator in receiver. Rotate flask to mix content thoroughly; then heat until all NH_3 is distilled.

6. Remove receiver, wash tip of condenser and titrate excess standard acid distilled with standard NaOH solution.

Calculation

$$\text{Protein(\%)} = \frac{(A-B) \times N \times 14.007 \times 6.25}{W}$$

Where	A	= volume (ml) of 0.2N HCl used sample titration
	B	= volume (ml) of 0.2N HCl used in blank titration
	N	= Normality of HCl
	W	= weight (g) of sample
	14.007	= atomic weight of nitrogen
	6.25	= the protein-nitrogen conversation factor for fish and its by-products

7.

Determination of ash content (AOAC, 2000) Method

1. Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burned off.
2. Cool the crucible in the desiccator (30 min).
3. Weigh the crucible and lid to 3 decimal places. Weigh about 5 g sample into the crucible. Heat over low Bunsen flame with lid half covered. When fumes are no longer produced, place crucible and lid in furnace.
2. Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after complete heating to prevent loss of fluffy ash. Cool down in the desiccator.
3. Weigh the ash with crucible and lid when the sample turns to gray. If not, return the crucible and lid to the furnace for the further ashing

Calculation

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$