CHAPTER-12

MINERAL NUTRITION

Introduction

All living organisms require macromolecules such as carbohydrates, fats, proteins, water, and minerals for growth and development. The important elements required in mineral nutrition include:

Methods to study the mineral requirements of plants

In 1860, Julius von Sachs, a prominent German botanist, demonstrated, for the first time, that plants could be grown to maturity in a defined nutrient solution in the complete absence of soil.

This technique of growing plants in a nutrient solution is known as hydroponics. Since then, several improvised methods have been employed to try and determine the mineral nutrients essential for plants. The essence of all these methods involves the culture of plants in a soil-free, defined mineral solution.
These methods require purified water and mineral nutrient salts.

After a series of experiments in which the roots of the plants were immersed in nutrient solutions and wherein an element was added/substituted/removed or given in varying concentrations, a mineral solution suitable for the plant growth was obtained. By this method, essential elements were identified and their deficiency symptoms were discovered.

Hydroponics has been successfully employed as a technique for the commercial production of vegetables such as tomato, seedless cucumber, and lettuce. It must be emphasized that the nutrient solutions must be adequately aerated to obtain optimum growth.

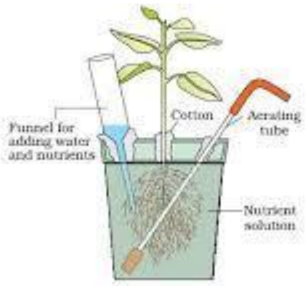
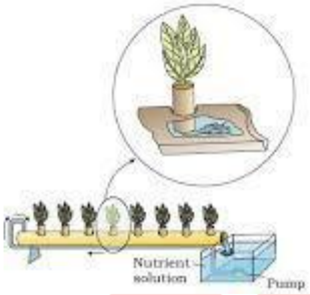


Figure 12.1 Diagram of a typical set-up for nutrient solution culture



Hydroponic plant production. Plants are grown in a tube or trough placed on a slight incline. A pump circulates a nutrient solution from a reservoir to the elevated end of the tube. The solution flows down the tube and returns to the reservoir due to gravity. Inset shows a plant whose roots are continuously bathed in an aerated nutrient solution. The arrows indicate the direction of the flow.

Essential mineral Elements

Mineral nutrition in plants is required for a variety of functions such as growth, repair, and photosynthesis. A deficiency of these essential elements can cause a variety of diseases that can range from chlorosis to necrosis. Elements like boron, calcium, chlorine, copper, iron, magnesium, molybdenum, manganese, nickel, phosphorus, potassium, sulfur, and zinc have been documented to have beneficial effects on the growth and sustenance of plants.

Most of the minerals present in soil can enter plants through roots. More than sixty elements of the 105 discovered so far are found in different plants. Some plant species accumulate selenium, some others gold, while some plants growing near nuclear test sites take up radioactive strontium.
Some techniques can detect the minerals even at a very low concentration (10-8 g/ mL). The question is, whether all the diverse mineral elements present in a plant, for example, gold and selenium as mentioned above, are really necessary for plants?

Criteria for Essentiality

The criteria for the essentiality of an element are given below:

- The element must be necessary for supporting normal growth and reproduction.
- In the absence of the element, the plants do not complete their life cycle or set the seeds.

- The requirement of the element must be specific and not replaceable by another element. In other words, deficiency of any one element cannot be met by supplying some other element.
- The element must be directly involved in the metabolism of the plant.

Based upon the above criteria only a few elements are essential for plant growth and metabolism. These elements are further divided into two broad categories based on their quantitative requirements.
(i) Macronutrients, and

(ii) Micronutrients

Macronutrients

Macronutrients are generally present in plant tissues in large amounts (more than 10 mmole Kg –1 of dry matter). The macronutrients include carbon, hydrogen, oxygen, nitrogen, phosphorous, sulphur, potassium, calcium, and magnesium. Of these, carbon, hydrogen, and oxygen are mainly obtained from CO2 and H2O, while the others are absorbed from the soil as mineral nutrition.

Micronutrients

Micronutrients or trace elements are needed in very small amounts (less than 10 mmole Kg –1 of dry matter). These include iron, manganese, copper, molybdenum, zinc, boron, chlorine, and nickel. In addition to the 17 essential elements named above, there are some beneficial elements such as sodium, silicon, cobalt, and selenium. They are required by higher plants.

Essential elements can also be grouped into four broad categories based on their diverse functions.

These categories are:
(i) Essential elements as components of biomolecules and hence structural elements of cells (e.g., carbon, hydrogen, oxygen, and nitrogen).

(ii) Essential elements that are components of energy-related chemical compounds in plants (e.g., magnesium in chlorophyll and phosphorous in ATP).

(iii) Essential elements that activate or inhibit enzymes, for example, Mg2+ is an activator for both ribulose bisphosphate carboxylase oxygenase and phosphoenolpyruvate carboxylase, both of which are critical enzymes in photosynthetic carbon fixation; Zn2+ is an activator of alcohol dehydrogenase and Mo of nitrogenase during nitrogen metabolism.

(iv) Some essential elements can alter the osmotic potential of a cell. Potassium plays an important role in the opening and closing of stomata.

Functions of Mineral Nutrients

The mineral nutrients perform the following functions:

- Carbon, hydrogen, and oxygen enter into the cell wall and protoplasm and form the plant body.
- The minerals present in the cell sap maintain the osmotic pressure of the cell.
- Calcium, sodium, and potassium maintain the permeability of the cell membrane.
- The cations and anions affect the pH of the cell sap.
- A few salts and minerals balance the harmful effect of other nutrients.
- Several elements act as a catalyst for biochemical reactions.

Mineral Nutrition is defined as the naturally occurring inorganic nutrient found in the soil and food that is essential for the proper functioning of animal and plant bodies. Minerals are vital elements necessary for the body. Both plants and animals require minerals essentially. For example, zinc is necessary for the manufacture of protein and cell division.

Role of Micro and macronutrients

- **Balancing function:** Some salts or minerals act against the harmful effects of the other nutrients thus balancing each other.
- **Maintenance of osmotic pressure:** Several minerals cell sap is present in the organic or inorganic form to regulate the organic pressure of the cell.
- **Influencing the pH of the cell sap:** Different anions and cations influences the pH of the cell sap.
- **Construction of the plant body:** Carbon, Hydrogen, and Oxygen are elements that help to construct the plant body by entering protoplasm and constitution of the wall.
- **Catalysis of the biochemical reaction:** Certain elements like zinc, magnesium, calcium, and copper act as metallic catalysts in biochemical reactions.
- **Effects of Toxicity:** Certain minerals like arsenic and copper has a toxic effect on the protoplasm under specific conditions.

Various forms and functions of essential nutrient elements are given below.

Nitrogen: This is the essential nutrient element required by plants in the greatest amount. It is absorbed mainly as NO_3^- though some are also taken up as NO_2^- or NH_4^+. Nitrogen is required by all parts of a plant, particularly the meristematic tissues and the metabolically active cells. Nitrogen is one of the major constituents of proteins, nucleic acids, vitamins, and hormones.

Phosphorus: Phosphorus is absorbed by the plants from the soil in the form of phosphate ions (either as $H_2 PO^{4-}$ or HPO_4^{2-}). Phosphorus is a constituent of cell membranes, certain proteins, all nucleic acids, and nucleotides, and is required for all phosphorylation reactions.

Potassium: It is absorbed as a potassium ion (K^+). In plants, this is required in more abundant quantities in the meristematic tissues, buds, leaves, and root tips. Potassium helps to maintain an anion-cation balance in cells and is involved in protein synthesis, opening and closing of stomata, activation of enzymes, and in the maintenance of the turgidity of cells.

Calcium: Plant absorbs calcium from the soil in the form of calcium ions (Ca2+). Calcium is required by meristematic and differentiating tissues. During cell division, it is used in the synthesis of cell walls, particularly as calcium pectate in the middle lamella. It is also needed during the formation of the mitotic spindle. It accumulates in older leaves. It is involved in the normal functioning of the cell membranes. It activates certain enzymes and plays an important role in regulating metabolic activities. Magnesium: It is absorbed by plants in the form of divalent Mg^{2+}. It activates the enzymes of respiration, photosynthesis, and are involved in the synthesis of DNA and RNA.

Magnesium is a constituent of the ring structure of chlorophyll and helps to maintain the ribosome structure. Sulphur: Plants obtain sulphur in the form of sulphate (SO_4^{2-}) .

Sulphur is present in two amino acids – cysteine and methionine and is the main constituent of several coenzymes, vitamins (thiamine, biotin, Coenzyme A), and ferredoxin.

Iron: Plants obtain iron in the form of ferric ions (Fe^{3+}). It is required in larger amounts in comparison to other micronutrients. It is an important constituent of proteins involved in the transfer of electrons like ferredoxin and cytochromes. It is reversibly oxidized from Fe2+ to Fe3+ during electron transfer. It activates the catalase enzyme and is essential for the formation of chlorophyll.

Manganese: It is absorbed in the form of manganous ions (Mn^{2+}). It activates many enzymes involved in photosynthesis, respiration, and nitrogen metabolism. The best-defined function of manganese is in the splitting of water to liberate oxygen during photosynthesis.

Zinc: Plants obtain zinc as Zn^{2+} ions. It activates various enzymes, especially carboxylases. It is also needed in the synthesis of auxin.

Copper: It is absorbed as cupric ions (Cu^{2+}). It is essential for the overall metabolism in plants. Like iron, it is associated with certain enzymes involved in redox reactions and is reversibly oxidized from Cu^{+} to Cu^{2+} .

Boron : It is absorbed as BO_3^{3-} or $B_4O_7^{2-}$.

Boron is required for uptake and utilization of Ca^{2+}, membrane functioning, pollen germination, cell elongation, cell differentiation, and carbohydrate translocation.

Molybdenum: Plants obtain it in the form of molybdate ions (MoO_2^{2+}). It is a component of several enzymes, including nitrogenase and nitrate reductase both of which participate in nitrogen metabolism.

Chlorine: It is absorbed in the form of chloride anion (Cl^{-}). Along with Na^{+} and K^{+}, it helps in determining the solute concentration and the anioncation balance in cells. It is essential for the water-splitting reaction in photosynthesis, a reaction that leads to oxygen evolution.

Micronutrients

Functions of some of the Micronutrients are stated below:

Copper

- It is a component of oxidase, cytochrome oxidase, phenolases, and ascorbic acid oxidase that is responsible for activating the enzymes.
- Copper plays a vital role in photophosphorylation.
- It also helps to balance carbohydrate-nitrogen regulation.

Manganese

- It is necessary for photosynthesis during the photolysis of water.
- The mineral is required for the synthesis of chlorophyll.
- It acts as an activator of nitrogen metabolism.

Zinc

- It is essential for the synthesis of tryptophan, metabolism of carbohydrates, and phosphorus.
- It is a constituent of enzymes like alcohol dehydrate-gas, carbonic anhydrase, lactic dehydrogenase, hexokinase, and carboxypeptidase.

Macronutrients

Functions of certain macronutrients are stated below:

Phosphorous

- Phosphorous boosts fruit ripening and root growth healthily by helping the translocation of carbohydrates.
- They are found abundantly in fruits and seeds.
- Deficiency of Phosphorus leads to the premature fall of leaves and they turn purplish or dark green.

Nitrogen

- It is present in various coenzymes, hormones, and ATP, etc.
- Nitrogen is a vital constituent of vitamins, nucleic acids, proteins, and many others.
- Deficiency of nitrogen leads to the complete suppression of flowering and fruiting, impaired growth, and development of anthocyanin pigmentation in stems.

Potassium

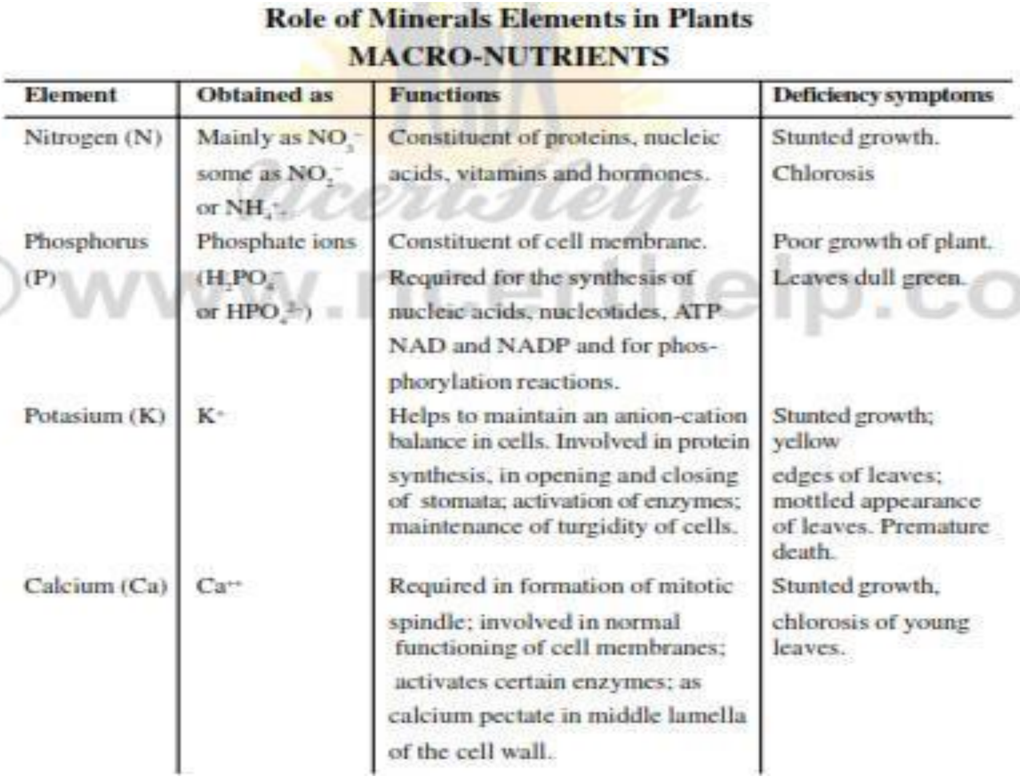
Potassium is the only monovalent cation that is necessary for plants. It acts as an enzyme activator including DNA polymerase. The deficiency of potassium leads to Mottled chlorosis.

Role of Macro and Micronutrients

The following are the major functions of macro and micronutrients:

Mineral Nutrients	Functions
Nitrogen	An important constituent of nucleic acid, protein, hormones, and vitamins
Phosphorus	Promotes root growth and fruit ripening
Potassium	It acts as an activator for several enzymes
Calcium	Facilitates the formation of the middle lamella of plants and acts as an enzyme activator
Magnesium	Plays a vital role in the metabolism of carbohydrates, lipids

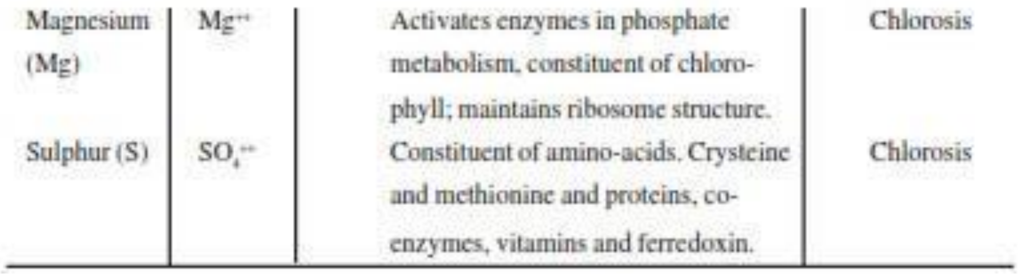
Sulphur	The major, constituent of amino acids and vitamins
Iron	Plays an important role in the energy conversion reaction reactions of respiration and photosynthesis, activates nitrate reductase and aconitase
Manganese	Essential for chlorophyll synthesis, initiate photolysis of water
Copper	Plays an important role in photophosphorylation
Molybdenum	It helps in the synthesis of ascorbic acid
Chlorine	Helps in the photolysis of water in photosystem-II



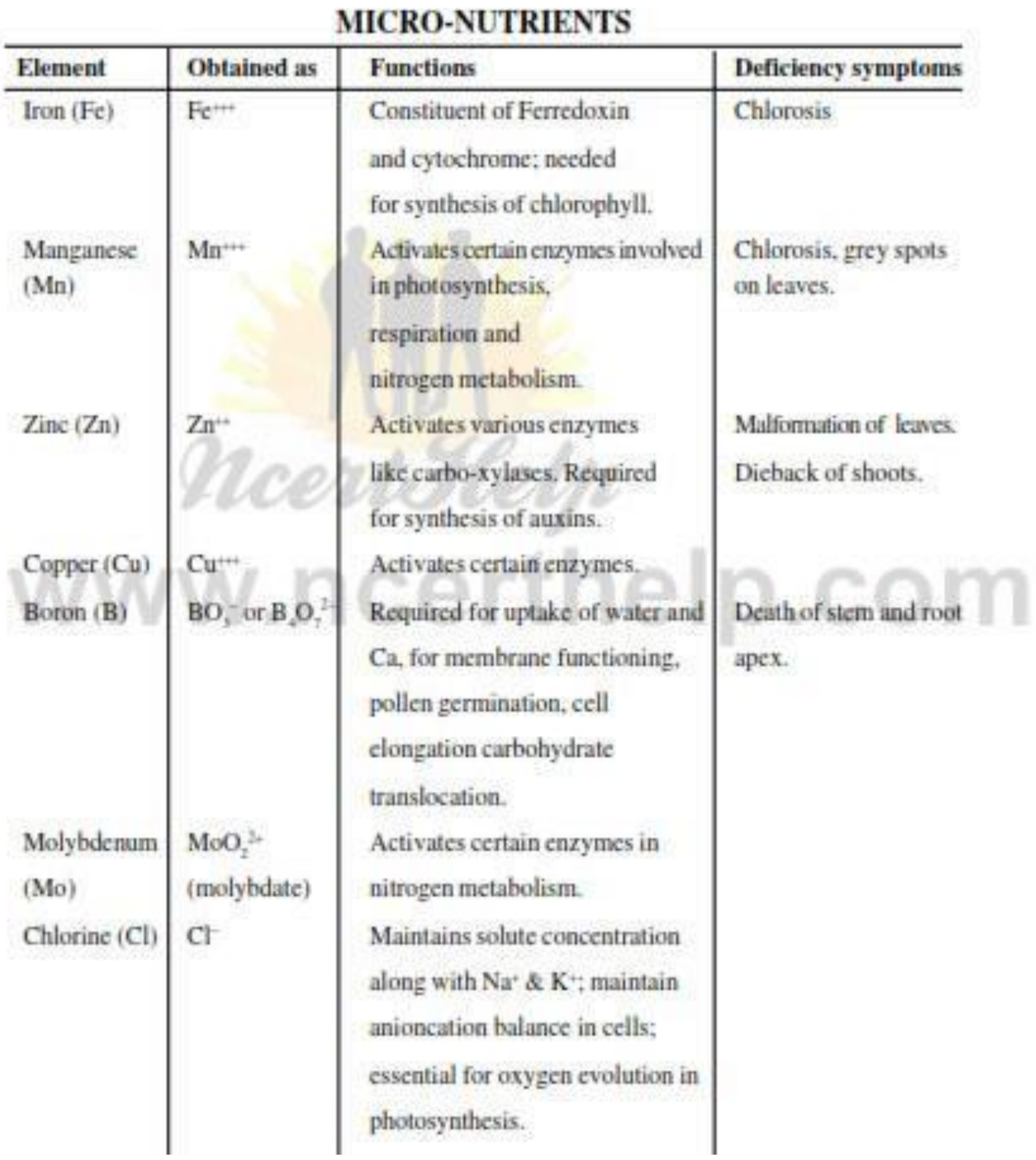
Role of Minerals Elements in Plants

MACRO-NUTRIENTS

Element	Obtained as	Functions	Deficiency symptoms
Nitrogen (N)	Mainly as NO_3^- some as NO_2^- or NH_4^+.	Constituent of proteins, nucleic acids, vitamins and hormones.	Stunted growth. Chlorosis
Phosphorus (P)	Phosphate ions ($H_2PO_4^-$ or HPO_4^{2-})	Constituent of cell membrane. Required for the synthesis of nucleic acids, nucleotides, ATP NAD and NADP and for phosphorylation reactions.	Poor growth of plant. Leaves dull green.
Potasium (K)	K^+	Helps to maintain an anion-cation balance in cells. Involved in protein synthesis, in opening and closing of stomata; activation of enzymes; maintenance of turgidity of cells.	Stunted growth; yellow edges of leaves; mottled appearance of leaves. Premature death.
Calcium (Ca)	Ca^{++}	Required in formation of mitotic spindle; involved in normal functioning of cell membranes; activates certain enzymes; as calcium pectate in middle lamella of the cell wall.	Stunted growth, chlorosis of young leaves.



Magnesium (Mg)	Mg^{++}	Activates enzymes in phosphate metabolism, constituent of chlorophyll; maintains ribosome structure.	Chlorosis
Sulphur (S)	SO$_4^{++}$	Constituent of amino-acids. Crysteine and methionine and proteins, co-enzymes, vitamins and ferredoxin.	Chlorosis



MICRO-NUTRIENTS

Element	Obtained as	Functions	Deficiency symptoms
Iron (Fe)	Fe^{+++}	Constituent of Ferredoxin and cytochrome; needed for synthesis of chlorophyll.	Chlorosis
Manganese (Mn)	Mn^{+++}	Activates certain enzymes involved in photosynthesis, respiration and nitrogen metabolism.	Chlorosis, grey spots on leaves.
Zinc (Zn)	Zn^{++}	Activates various enzymes like carbo-xylases. Required for synthesis of auxins.	Malformation of leaves. Dieback of shoots.
Copper (Cu)	Cu^{+++}	Activates certain enzymes.	
Boron (B)	BO$_3^-$ or B$_4$O$_7^{2-}$	Required for uptake of water and Ca, for membrane functioning, pollen germination, cell elongation carbohydrate translocation.	Death of stem and root apex.
Molybdenum (Mo)	MoO$_2^{2+}$ (molybdate)	Activates certain enzymes in nitrogen metabolism.	
Chlorine (Cl)	Cl$^-$	Maintains solute concentration along with Na$^+$ & K$^+$; maintain anioncation balance in cells; essential for oxygen evolution in photosynthesis.	

Deficiency symptoms of essential elements

Whenever the supply of an essential element becomes limited, plant growth is retarded. The concentration of the essential element below which plant growth is retarded is termed as

critical concentration. The element is said to be deficient when present below the critical concentration.

Since each element has one or more specific structural or functional role in plants, in the absence of any particular element, plants show certain morphological changes. These morphological changes are indicative of certain element deficiencies and are called deficiency symptoms.

The deficiency symptoms vary from element to element and they disappear when the deficient mineral nutrient is provided to the plant.

However, if deprivation continues, it may eventually lead to the death of the plant. The parts of the plants that show the deficiency symptoms also depend on the mobility of the element in the plant. For elements that are actively mobilized within the plants and exported to young developing tissues, the deficiency symptoms tend to appear first in the older tissues.

For example, the deficiency symptoms of nitrogen, potassium, and magnesium are visible first in the senescent leaves. In the older leaves, biomolecules containing these elements are broken down, making these elements available for mobilizing to younger leaves.

The deficiency symptoms tend to appear first in the young tissues whenever the elements are relatively immobile and are not transported out of the mature organs, for example, elements like sulphur and calcium are a part of the structural component of the cell and hence are not easily released. This aspect of the mineral nutrition of plants is of great significance and importance to agriculture and horticulture.

The kind of deficiency symptoms shown in plants includes chlorosis, necrosis, and stunted plant growth, premature fall of leaves and buds, and inhibition of cell division. Chlorosis is the loss of chlorophyll leading to yellowing in leaves. This symptom is caused by the deficiency of elements N, K, Mg, S, Fe, Mn, Zn, and Mo. Likewise, necrosis, or death of tissue, particularly leaf tissue, is due to the deficiency of Ca, Mg, Cu, K. Lack or low level of N, K, S, Mo causes inhibition of cell division. Some elements like N, S, Mo delay flowering if their concentration in plants is low.

The deficiency of any element can cause multiple symptoms and that the same symptoms may be caused by the deficiency of one of several different elements.

Hence, to identify the deficient element, one has to study all the symptoms developed in all the various parts of the plant and compare them with the available standard tables.

The deficiency of major mineral nutrients is given below:

Mineral Nutrients	**Deficiency Symptoms**
Nitrogen	Impaired plant growth, chlorosis, delayed flowering and fruiting
Phosphorus	Premature leaf fall, necrosis

Sulphur	Delayed flowering and fruiting, premature leaf fall
Potassium	Mottled chlorosis, inhibition of protein synthesis and photosynthesis
Calcium	Chlorosis, distortion of leaf shape
Magnesium	Interveinal chlorosis, depression of internal phloem
Iron	Chlorosis, inhibition of protein synthesis and chloroplast formation
Chlorine	Wilting of leaves, brown edges, leaf spots
Copper	Causes “dieback” disease in leaves, Reduction in vegetative and reproductive growth

Toxicity of Micronutrients

The requirement of micronutrients is always in low amounts while their moderate decrease causes the deficiency symptoms and a moderate increase causes toxicity. In other words, there is a narrow range of concentration at which the elements are optimum. Any mineral ion concentration in tissues that reduces the dry weight of tissues by about 10 per cent is considered toxic. Such critical concentrations vary widely among different micronutrients. The toxicity symptoms are difficult to identify. Toxicity levels for any element also vary for different plants. Many times, excess of an element may inhibit the uptake of another element. For example, the prominent symptom of manganese toxicity is the appearance of brown spots surrounded by chlorotic veins. It is important to know that manganese competes with iron and magnesium for uptake and with magnesium for binding with enzymes. Manganese also inhibits calcium translocation in the shoot apex. Therefore, excess of manganese may induce deficiencies of iron, magnesium, and calcium. Thus, what appears as symptoms of manganese toxicity may be the deficiency symptoms of iron, magnesium, and calcium.

Mechanism of absorption of elements

Much of the studies on the mechanism of absorption of elements by plants have been carried out in isolated cells, tissues, or organs. These studies revealed that the process of absorption can be demarcated into two main phases. In the first phase, the initial rapid uptake of ions into the ‘free space’ or ‘outer space’ of cells – the apoplast, is passive. In the second phase of uptake, the ions are taken in slowly into the ‘inner space’ – the symplast of the cells. The passive movement of ions into the apoplast usually occurs through ion-channels, the transmembrane proteins that function as selective pores. On the other hand, the entry or exit of ions to and from the symplast requires the expenditure of metabolic energy, which is an active process. The movement of ions is usually called flux; the inward movement into the cells is influx and the outward movement, efflux.

Translocation of solutes

Mineral salts are translocated through xylem along with the ascending stream of water, which is pulled up through the plant by the transpirational pull. Analysis of xylem sap shows the presence of mineral salts in it. The use of radioisotopes of mineral elements also substantiates the view that they are transported through the xylem.

Soil as a reservoir of essential elements

The majority of the nutrients that are essential for the growth and development of plants become available to the roots due to weathering and breakdown of rocks. These processes enrich the soil with dissolved ions and inorganic salts. Since they are derived from the rock minerals, their role in plant nutrition is referred to as mineral nutrition. Soil consists of a wide variety of substances. Soil not only supplies minerals but also harbours haemoglobin nitrogen-fixing bacteria, other microbes, holds water, supplies air to the roots, and acts as a matrix that stabilizes the plant.

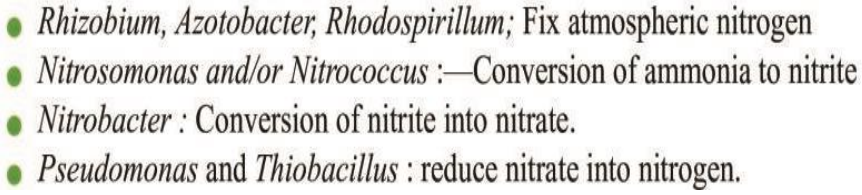
Since deficiency of essential minerals affects the crop yield, there is often a need for supplying them through fertilizers. Both macro-nutrients (N, P, K, S, etc.) and micro-nutrients (Cu, Zn, Fe, Mn, etc.) form components of fertilizers and are applied as per need.

Metabolism of nitrogen

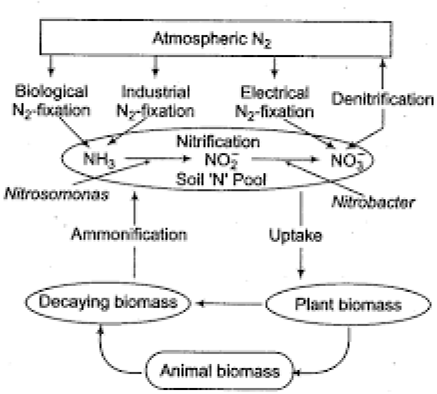
Nitrogen cycle

Apart from carbon, hydrogen, and oxygen, nitrogen is the most prevalent element in living organisms. Nitrogen is a constituent of amino acids, proteins, hormones, chlorophylls, and many of the vitamins. Plants compete with microbes for the limited nitrogen that is available in the soil. Thus, nitrogen is a limiting nutrient for both natural and agricultural ecosystems.

Nitrogen exists as two nitrogen atoms joined by a very strong triple covalent bond (N = N). The process of conversion of nitrogen (N_2) to ammonia is termed as nitrogen fixation. In nature, lightning and ultraviolet radiation provide enough energy to convert nitrogen to nitrogen oxides (NO, NO_2, N_2O). Industrial combustions, forest fires, automobile exhausts, and power-generating stations are also sources of atmospheric nitrogen oxides.



- *Rhizobium, Azotobacter, Rhodospirillum;* Fix atmospheric nitrogen
- *Nitrosomonas and/or Nitrococcus* :—Conversion of ammonia to nitrite
- *Nitrobacter* : Conversion of nitrite into nitrate.
- *Pseudomonas* and *Thiobacillus* : reduce nitrate into nitrogen.



Nitrogen cycle

The nitrogen cycle showing the relationship between the three main nitrogen pools – atmospheric
soil, and biomass.

The decomposition of organic nitrogen of dead plants and animals into ammonia is called ammonification. Some of these ammonia volatilizes and re-enters the atmosphere but most of it is converted into nitrate by soil bacteria in the following steps:

$$2NH_3 + 3O_2 \rightarrow 2NO_2^- + 2H^+ + 2H_2O \quad \ldots(i)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad \ldots(ii)$$

Ammonia is first oxidized to nitrite by the bacteria *Nitrosomonas* and/or *Nitrococcus*. The nitrite is further oxidized to nitrate with the help of the bacterium *Nitrobacter*. These steps are called nitrification. These nitrifying bacteria are chemoautotrophs.

The nitrate thus formed is absorbed by plants and is transported to the leaves. In leaves, it is reduced to form ammonia that finally forms the amine group of amino acids. Nitrate present in the soil is also reduced to nitrogen by the process of denitrification. Denitrification is carried by bacteria *Pseudomonas* and *Thiobacillus*.

Thus nitrogen cycle moves through the utilize following processes.

Nitrogen fixation—The process of conversion of Nitrogen (N_2) into ammonia (NH_3).

Ammonification—The process of decomposition of organic nitrogen of plants and animals (proteins) into ammonia.

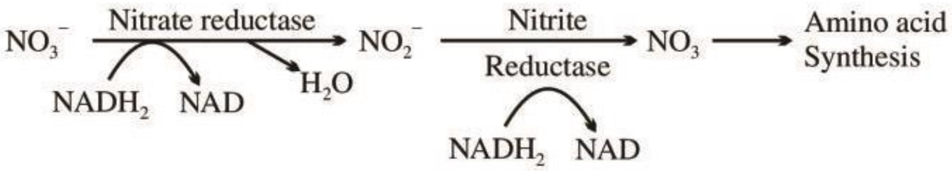
$$\text{Protien in Dead Plants and Animals} \xrightarrow[\text{Bacteria}]{\text{Ammonifying}} NH_3$$

Nitrification—The ammonia so formed may volatilise and re-enter the atmosphere, or some of the ammonia may be converted first into nitrite and then into nitrate by soil bacteria

$$2NH_3 + 3O_2 \xrightarrow{\text{Nitrosomonas}} 2NO_2^- + 2H_2O + 2H^+$$

$$2NO_2^- + O_2 \xrightarrow{\text{Nitrobactor}} 2NO_3^-$$

The Nitrate so formed can be easily absorbed by the plants and transported to leaves. In leaves, nitrate is reduced to ammonia to form amino-acids, because nitrate can not used by plants as such.



Denitrification—Process of reduction of the nitrate present in soil to nitrogen. It is carried out by bacteria like *Pseudomonas* and *Thiobacillus*.

Biological Nitrogen Fixation—Reduction of nitrogen to ammonia by living organisms. Certain prokaryotes are able to fix nitrogen because of presence of 'nitrogenase' enzyme in them.

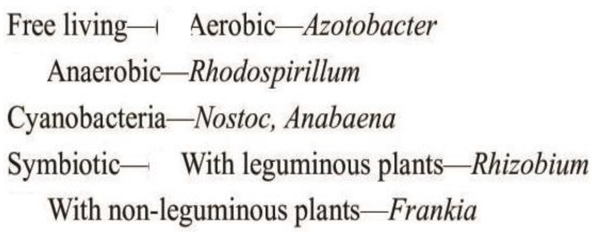
Biological Nitrogen Fixation

Very few living organisms can utilize the nitrogen in the form N_2, available abundantly in the air. Only certain prokaryotic species are capable of fixing nitrogen. The reduction of nitrogen to ammonia by living organisms is called biological nitrogen fixation. The enzyme, nitrogenase which is capable of nitrogen reduction is present exclusively in prokaryotes. Such microbes are called N_2- fixers.

nitrogenase

$N \equiv N$ NH_3

The nitrogen-fixing microbes could be free-living or symbiotic. Examples of free-living nitrogen-fixing aerobic microbes are *Azotobacter* and *Beijerinckia* while *Rhodospirillum* is anaerobic and free-living. Also, some cyanobacteria such as *Anabaena* and *Nostoc* are free-living nitrogen-fixers.



Free living— Aerobic—*Azotobacter*
Anaerobic—*Rhodospirillum*
Cyanobacteria—*Nostoc, Anabaena*
Symbiotic— With leguminous plants—*Rhizobium*
With non-leguminous plants—*Frankia*

Symbiotic biological nitrogen fixation

Several types of symbiotic biological nitrogen-fixing associations are known. The most prominent among them is the legume-bacteria relationship. Species of rod-shaped *Rhizobium* have such a relationship with the roots of several legumes such as alfalfa, sweet clover, sweet pea, lentils, garden pea, broad bean, clover beans, etc. The most common association on roots is as nodules. These nodules are small outgrowths on the roots. The microbe, *Frankia,* also produces nitrogen-fixing nodules on the roots of non-leguminous plants (e.g., *Alnus*). Both *Rhizobium* and *Frankia* are free-living in soil, but as symbionts, can fix atmospheric nitrogen.

The near-spherical outgrowths on the roots are called nodules. The nodules appear pink due to the presence of leguminous haemoglobin or leg-haemoglobin.

Nodule Formation

Nodule formation involves a sequence of multiple interactions between *Rhizobium* and the roots of the host plant.

Principal stages in the nodule formation are summarised as follows:

Rhizobia multiply and colonize the surroundings of roots and get attached to epidermal and root hair cells. The root-hairs curl and the bacteria invade the root-hair.
An infection thread is produced carrying the bacteria into the cortex of the root, where they initiate the nodule formation in the cortex of the root.
Then the bacteria are released from the thread into the cells which leads to the differentiation of specialized nitrogen-fixing cells. The nodule thus formed, establishes a direct vascular connection with the host for the exchange of nutrients.

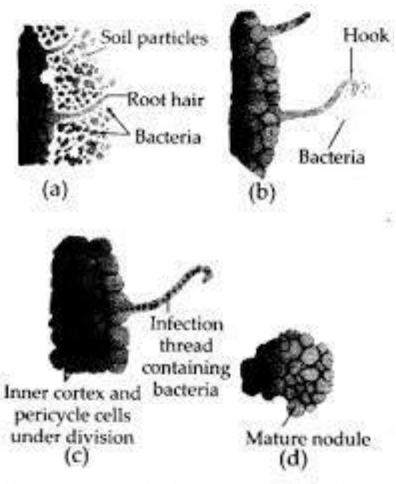
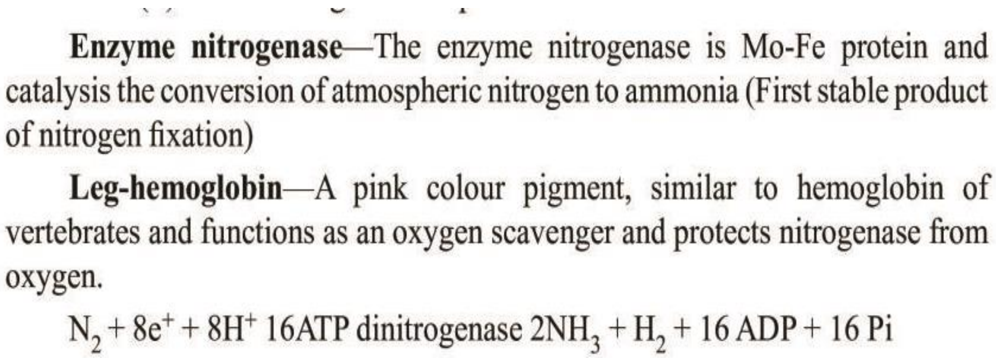


Fig.: Development of root nodule.

Development of root nodules in soybean : (a) *Rhizobium* bacteria contact a susceptible root hair, divide near it, (b) Successful infection of the root hair causes it to curl, (c) Infected thread carries the bacteria to the inner cortex. The bacteria get modified into rod-shaped bacteroids and cause inner cortical and pericycle cells to divide. Division and growth of cortical and pericycle cells lead to nodule formation, (d) A mature nodule is complete with vascular tissues continuous with those of the root.

The reaction is as follows:



Enzyme nitrogenase—The enzyme nitrogenase is Mo-Fe protein and catalysis the conversion of atmospheric nitrogen to ammonia (First stable product of nitrogen fixation)

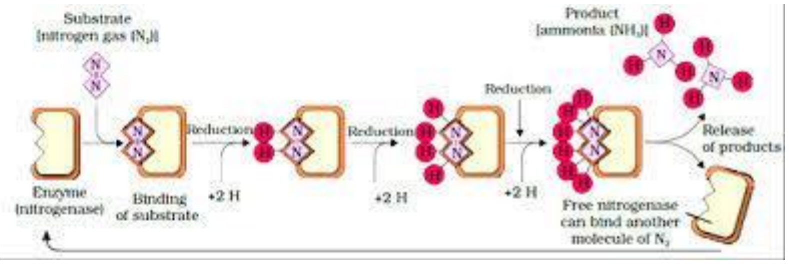
Leg-hemoglobin—A pink colour pigment, similar to hemoglobin of vertebrates and functions as an oxygen scavenger and protects nitrogenase from oxygen.

$N_2 + 8e^+ + 8H^+$ 16ATP dinitrogenase $2NH_3 + H_2 + 16$ ADP + 16 Pi

– $\longrightarrow$

The enzyme nitrogenase is highly sensitive to the molecular oxygen; it requires anaerobic conditions. The nodules have adaptations that ensure that the enzyme is protected from oxygen. To protect these enzymes, the nodule contains an oxygen scavenger called leg-haemoglobin. It is interesting to note that these microbes live as aerobes under free-living conditions (where nitrogenase is not operational), but during nitrogen-fixing events, they become anaerobic (thus protecting the nitrogenase enzyme).

The reaction that the ammonia synthesis by nitrogenease requires a very high input of energy (8 ATP for each NH_3 produced). The energy required, thus, is obtained from the respiration of the host cells.



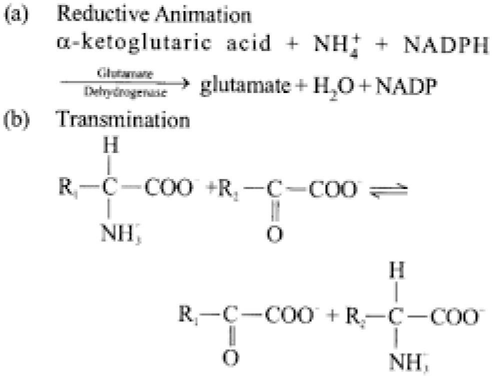
Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found in nitrogen-fixing bacteria.

Fate of ammonia

At physiological pH, the ammonia is protonated to form NH_4 (ammonium) ion. While most of the plants can assimilate nitrate as well as ammonium ions, the latter is quite toxic to plants and hence cannot accumulate in them.

NH^{4} $^{+}$ is used to synthesize amino acids in plants.

There are two main ways in which this can take place:
(i) Reductive amination: In these processes, ammonia reacts with a-ketoglutaric acid and forms glutamic acid as indicated in the



(a) Reductive Animation

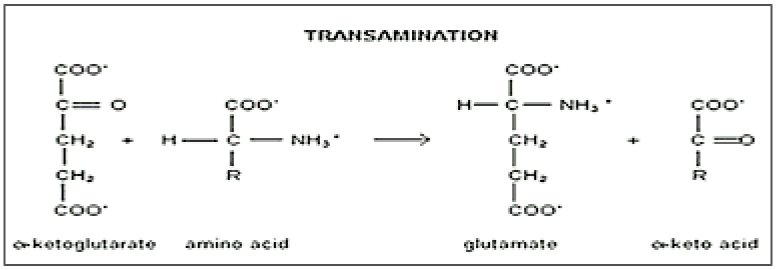
$$\alpha\text{-ketoglutaric acid} + NH_4^+ + NADPH \xrightarrow[\text{Dehydrogenase}]{\text{Glutamate}} \text{glutamate} + H_2O + NADP$$

(b) Transmination

$$R_1-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle NH_3^+}{|}}{C}}-COO^- + R_2-\underset{\underset{\displaystyle O}{\|}}{C}-COO^- \rightleftharpoons R_1-\underset{\underset{\displaystyle O}{\|}}{C}-COO^- + R_2-\overset{\overset{\displaystyle H}{|}}{\underset{\underset{\displaystyle NH_3^+}{|}}{C}}-COO^-$$

(ii)Transamination: It involves the transfer of amino group from one amino acid to the keto group of a keto acid. Glutamic acid is the main amino acid from which the transfer of NH_2, the amino group takes place and other amino acids are formed through transamination. The enzyme transaminase catalyzes all such reactions.

For example,



The two most important amides – asparagine and glutamine – found in plants are a structural part of proteins. They are formed from two amino acids, namely aspartic acid and glutamic acid, respectively, by the addition of another amino group to each. The hydroxyl part of the acid is replaced by another NH_2 – radicle.

Since amides contain more nitrogen than the amino acids, they are transported to other parts of the plant via xylem vessels. Also, along with the transpiration stream the nodules of some plants (e.g., soybean) export the fixed nitrogen as ureides. These compounds also have synthesized particularly high nitrogen to carbon ratio.

Important terms

Autotroph
An organism that synthesizes its required nutrients from simple and inorganic substances.

Heterotroph
An organism that cannot synthesize its nutrients and depend on others.

Necrosis
Death of cells and tissues.

Biological nitrogen fixation
Conversion of atmospheric into organic compounds by living organisms. Nitrification: Conversion of ammonia (NH) into nitrite and then to nitrate. 3

Denitrification
A process of conversion of nitrate into nitrous oxide and nitrogen gas (N).

Leg-hemoglobin
A pinkish pigment found in the root nodules of legumes. 2 It acts as an oxygen scavenger and protects the nitrogenase.

Flux
The movement of ions is called flux. Necrosis: Death of tissues particularly leaf tissue due to deficiency of Ca, Mg, Cu, K. Mineral

Nutrition
Plants require minerals elements for their growth and development. The utilization of various absorbed ions by a plant for growth and development is called mineral nutrition of the plant.

Hydroponics
The soil-less culture of plants, where roots are immersed in a nutrient solution without soil is called hydroponics. The result obtained from hydroponics may be used to determine deficiency symptoms of essential elements.