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S.Y. B.Sc. (Sem. IV) (CBCS)

MICROBIOLOGY

[401]: APPLIED AND ENVIRONMENTAL MICROBIOLOGY

Unit 1
SOIL MICROBIOLOGY

Prepared By

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SOIL MICROBIOLOGY

- Soil is a complex environment offering a variety of microhabitats.
- This is one reason why **microbial diversity** in soils is much greater than that found in aquatic environments.
- In terrestrial ecosystems, primary production is performed by plants, but the nutrient recycling that occurs though a microbial loop is also essential. Each climate and soil type has a community of microbes specifically adapted to that particular microhabitat.
- Many microbes inhabit the pores between soil particles; others live in association with plants. The plant root surface **(rhizoplane**) and the region close to plant roots (**the rhizosphere)** are important sites for microbial growth.
- **Mycorrhizal fungi** associate with most plants. In this relationship, the fungi provide their plant partner with essential nutrients like nitrogen and phosphorus, while the plant supplies organic carbon to the fungi.
- Rhizobia include ex-**13-proteobacteria** that form nodules within the roots of leguminous plants, where they fix nitrogen. This process has been best studied in the genus ***Rhizobium*** and its relatives; it involves a complex plant-microbe communication system and the differentiation of the bacterium into a form that can fix nitrogen. A variety of other bacteria, including the **actinomycete *Frankia*,** also fix nitrogen while interacting with plants.
- The plant **pathogen *Agrobacterium*** also relies on an intercellular communication system with host plants, in which it causes tumors called galls. These arise following the insertion of a fragment of bacterial DNA, called T DNA, into the plant cell's chromosome.
- **Subsurface microbiology** is a relatively new and exciting field that explores vast microbial communities living deep beneath the top- soil. Recent studies show that the biomass within this microbial world equals at least one-third of that living above ground**.**
- The soil is composed of five major components**: mineral matter, water, air, organic matter and living organisms.**
- The quantity of these constituents is not the same in all soils but, varies with the locality. The inorganic portion of the soil, because its influence on nutrient availability, aeration, and water retention has a marked effect on the microbial inhabitants.
- The soil is not a dead inert material. Actually, it is full of life. **One gram of soil** contains approximately **one million microorganisms**. Man depends upon the soil for his food.
- The soil depends upon the microorganisms for its fertility. The soil is not **a static medium**. The soil is a tremendous growth medium. The soil has organic matter soil solution and soil air. All these components are affected by the activities of microorganisms.
- So, the soil is constantly changing medium. The soil solution in agricultural soil has ions like

K^+, Na^+, Mg^{++}, Ca^{++}, Fe^+, S^-, NO_3^- SO^-, PO and others. These ions are very essential in culture media.

- In a fertile soil, these elements in mineral form are supplemented by organic compounds derived from the decomposition of animal and plant residues. Thus, the soil is an ***excellent natural medium* for microorganisms.**

Sr.No.	QUESTION	ANSWER
1	At which place microbial diversity is present in large amount?	Soil
2	region close to plant roots is known as which name?	Rhizosphere
3	Which fungi associate with most plants ?	Mycorrhizal fungi
4	The soil is composed of which five major components?	mineral matter, water, air, organic matter and living organisms
5	____ is an *excellent natural medium* for microorganisms.	Soil

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Components of Soil

- Soil is an admixture *of* **five major components** viz. organic matter, mineral matter, soil-air, soil water and soil microorganisms/living organisms. The amount/ proposition of these components varies with locality and climate.

1. **Mineral / Inorganic Matter**: It is derived from parent rocks/bed rocks through decomposition, disintegration and weathering process. Different types of inorganic compounds containing various minerals are present in soil. Amongst them the dominant minerals are **Silicon, Aluminum and iron** and others like **Carbon, Calcium Potassium, Manganese, Sodium, Sulphur, Phosphorus** etc. are in trace amount. The proportion of mineral matter in soil is slightly less than half of the total volume of the soil.

2. **Organic matter/components:**

- Derived from organic residues **of plants and animals** added in the soil.
- Organic matter serves not only as a source of food for microorganisms but also supplies energy for the vital processes of metabolism which are characteristics of all living organisms.
- Organic matter in the soil is the potential source of **N, P and S** for plant growth.
- Microbial decomposition of organic matter releases the unavailable nutrients in available from.
- The proportion of organic matter in the soil ranges from 3-6% of the total volume of soil.

3. Soil Water:

- The amount of water present in soil varies considerably.
- Soil water comes from rain, snow, dew or irrigation.
- Soil water serves as a solvent and carrier of nutrients for the plant growth.
- The microorganisms inhabiting in the soil also require water for their metabolic activities.
- Soil water thus, indirectly affects plant growth through its effects on soil and microorganisms. Percentage of **soil-water is 25%** total volume of soil.

4. Soil air (Soil gases):

- A part of the soil volume which is not occupied by soil particles i.e., **pore spaces** are filled partly with soil water and partly with soil air.
- These two components **(water & air)** together only accounts for approximately half the soil's volume.
- Compared with atmospheric air, soil is lower in oxygen and higher in carbon dioxide, because CO2 is continuous recycled by the microorganisms during the process of decomposition of organic matter.
- Soil air comes from external atmosphere and contains nitrogen, oxygen Co2 and water vapor **(CO2 > oxygen).**
- Co2 in soil air **(0.3- 1.0%)** is more than atmospheric air **(0.03%).**
- Soil aeration plays important role in **plant growth, microbial population, and microbial activities in the soil.**

5. Soil microorganisms:

- Soil is an excellent culture media for the growth and development of various microorganisms.
- Soil is not an inert static material but a medium pulsating with life.
- Soil is now believed to be **dynamic** or living system.
- Soil contains several distinct groups of microorganisms and amongst them **bacteria, fungi, actinomycetes, algae, protozoa and viruses** are the most important. But bacteria are more numerous than any other kinds of microorganisms.
- Microorganisms form a very small fraction of the soil mass and occupy a volume of less than one percent.
- In the upper layer of soil (top soil up to 10-30 cm depth i.e., **Horizon A**), the microbial population is very high which decreases with depth of soil.
- Each organism or a group of organisms are responsible for a specific change / transformation in the soil.
- The final effect of various activities of microorganisms in the soil is to make the soil fit for the growth & development of higher plants.
- Living organisms present in the soil are grouped into two categories as follows.
    - Soil flora (micro flora) e.g**., Bacteria, fungi, Actinomycetes, Algae and**
    - Soil fauna (micro fauna) animal like eg. **Protozoa, Nematodes, earthworms, moles, ants, rodents.**
- Relative proportion / percentage of various soil microorganisms are:
    - **Bacteria-aerobic (70%),**
    - **Bacteria anaerobic (13 %),**
    - **Actinomycetes (13%),**
    - **Fungi /molds (03 %) and**
    - **others (Algae Protozoa viruses) 0.2-0.8 %.**
- Soil organisms play key role in the nutrient transformations.

Sr. no.	QUESTION	ANSWER
1	Hoe many major constituent of soil?	Five
2	Which are dominant minerals present in soil?	Silicon, Aluminum and iron
3	Which source provide energy to plant material?	Organic matter
4	Which microorganism is dominant in soil?	Aerobic bacteria

Distribution of different types of soil microorganisms

- Soils contain five major groups of microorganisms. They are **bacteria, actinomycetes, fungi, algae and protozoa**. Among the soil microorganisms, bacteria are most dominant group of organisms.
- All kinds of bacteria are found in the soil. This is because all kinds of organic refuse are disposed off on the soil. Many of the soil bacteria perform useful functions like decomposition of organic matter, conversion of soil constituents into useful materials, production of antibiotics in the soil, and biogeochemical cycling of elements like **carbon, nitrogen, phosphorus, iron, sulfur and manganese.**
- The bacterial population of the soil exceeds the population of all other groups of microorganisms in both number and variety. Direct microscopic counts as high as several billions bacteria per a gram of soil have been reported.
- The actinomycetes population as many as millions per gram of soil is present. The most predominant genera present in the soil are ***Nocardia, Streptomyces*** **and** ***Micromonospora***. These organisms are responsible for the characteristic musty or earthy odor soon after the rainfall. This is due to sporulation of actinomycetes.
- **Actinomycetes** are capable of degrading many complex organic substances and con centration sequently play an important role in building soil fertility. The actinomycetes have ability to synthesize and excrete antibiotics. Most of the antibiotics are produced by actinomycetes. The presence of antibiotic substances in soil can be detected with great difficulty.
- The fungal population ranging from thousands to hundred thou- sands per gram of soil has been reported. They are aerobic in nature and found more numbers near the earth surface. They exist in the atmosphere as mycelial and spore stage.
- Fungi are active in decomposing the major constituents of **plant tissues, namely, cellulose, hemi-cellulose, lignin and pectin.**
- The population of algae in soil is very smaller than that of either bacteria or fungi. The major types present in the soil are the green algae and diatoms. Their photosynthetic nature accounts for their predominance on the surface or just below the surface layer of soil.

- In a fertile soil, biochemical activities of algae are masked by bacteria and fungi. In certain conditions, algae perform prominent and beneficial changes. For example, on barren and eroded lands they may initiate the accumulation of organic matter because of their ability to carry out photosynthesis and other metabolic activities.

- Many soil **protozoa are flagellates** or amoebas; the population per gram soil ranges from a few hundred to several thousand in moist soils rich in organic matter. Protozoa are of significance since their dominant mode of nutrition involves ingestion of bacteria.

Sr.no.	QUESTION	ANSWER
1	Which microorganisms present in soil?	Bacteria, fungi, protozoa, algae, actinomycetes
2	Which microorganism produces antibiotic?	Bacteria
3	Which microorganisms is responsible for musty smell after rain?	Nocardia, Streptomyces and Micromonospora
4	Plant and tissue are degraded by which microorganisms?	Fungi
5	Population of _____ is very small as compare to bacteria and fungi.	Algae

Rhizosphere

- The region which is adjacent to the root system is called rhizosphere. The microbial population on and around roots system considerably higher than that of root-free soil or non-rhizosphere soil.

- This may be due to the availability of nutrients from plant roots in the form of root nodules, secretions, lysates mucigel and sloughed off cells.

Rhizosphere effect

- **Bacteria predominate in rhizosphere soil and their growth is influenced by nutritional substances released from the plant tissues** e.g., amino acids, vitamins and other nutrients; the growth of the plant is influenced by the products of microbial metabolism that are released into the soil.

- It has been reported that amino acid requiring bacteria exist in the rhizosphere in larger numbers than in the root-free soil. It has been demonstrated that the micro flora of the rhizosphere is more active physiologically than that of non-rhizosphere soil.

- The rhizosphere effect improves the physiological conditions of the plant and ultimately result in higher yield. Greater rhizosphere effect is seen with bacteria (R:S ratio ranging from 10-20 times more) than with actinomycetes or fungi.

Phylosphere

- **The Dutch Microbiologist Ruinen** coined the term phyllosphere. The leaf surface has been termed as phylloplane and the zone on leaves inhabited by the microorganisms as phyllosphere.
- In forest vegetation, thick microbial epiphytic associations exist on leaves. The dominant and useful microorganisms on the leaf surfaces in the forest, vegetation happened to be nitrogen fixing bacteria such **as *Beijerinckia* and *Azotobacter***.
- Apart from these nitrogen fixing bacteria, other genera such as ***Pseudomonas, Pseudobacterium, Phytomonoas*** are also encountered on the leaf surface.

- The quantity and quality of phyllosphere organisms vary with the plant species and its morphological, physiological and environmental factors. The age of plant, its leaf spread, morphology and maturity level and the atmospheric factors greatly influence the phyllosphere microflora.

Soil Humus

- **Humus is the organic residue** in the soil resulting from decomposition of plant and animal residues in soil, or it is the highly complex organic residual matter in soil which is not readily degraded by microorganism, or it is the soft brown/dark colored amorphous substance composed of residual organic matter along with dead microorganisms.
- ***Composition of Humus:***
  - In most soil, percentage of humus ranges from 2-10 percent, whereas it is up to 90 percent in peat bog. On average humus is composed of Carbon (58 %), Nitrogen (3-6%, Av.5%), acids - humic acid, fulvic acid, humin, apocrenic acid, and C: N ratio 10:1 to 12:1.
  - During the course of their activities, the microorganisms synthesize number of compounds which plays important role in humus formation.
- ***Functions/Role of Humus:***
  - It improves physical condition of soil
  - Improve water holding capacity of soil
  - Serve as store house for essential plant nutrients
  - Plays important role in determining fertility level of soil

- It tends to make soils more granular with better aggregation of soil particles
- Prevent leaching losses of water-soluble plant nutrients
- Improve microbial/biological activity in soil and encourage better development of plant-root system in soil
- Act as buffering agent i.e., prevent sudden change in soil PH/soil reaction
- Serve as source of energy and food for the development of soil organisms
- It supplies both basic and acidic nutrients for the growth and development of higher plants
- Improves aeration and drainage by making the soil more porous

Soil Organisms

A. Soil Flora

- Microflora:
  1. Bacteria
  2. Fungi, Molds, Yeast, Mushroom
  3. Actinomycetes, Streptomyces
  4. Algae e.g., BGA, Yellow Green Algae, Golden Brown Algae.
- Macroflora: Roots of higher plants

B. Soil Fauna

a) Microfauna: Protozoa, Nematodes

b) Macrofauna: Earthworms. moles, ants & others.

As soil inhabit several diverse groups of microorganisms, but the most important amongst them are: bacteria, actinomycetes, fungi, algae and protozoa. The characteristics and their functions / role in the soil are described in the next topics.

Bacteria

- Most numerous in soil
- Most diverse metabolism
- Can be aerobic or anaerobic
- Optimal growth at pH 6-8

- Examples: *Nitrosomonas* and *Nitrobacter* in nitrification processes, N_2 fixers, fire blight is caused by a bacterium

Fungi

- Dominate the soil biomass
- Obligate aerobes
- Can survive desiccation
- Dominate in acid soils
- Negative impacts: Apple replant disease (*Rhizoctonia, Pythium, Fusarium*, and *Phytophthora*)
- Powdery mildew is caused by a fungus
- Beneficials: *Penicillium*
- There are species of fungi that trap harmful plant-parasitic nematodes.
- The mycorrhizae are fungi that live either on or in plant roots and act to extend the reach of root hairs into the soil.
- Mycorrhizae increase the uptake of water and nutrients, especially phosphorus.
- They are particularly important in degraded or less fertile soils. Roots colonized by mycorrhizae are less likely to be penetrated by root-feeding nematodes, since the pest cannot pierce the thick fungal network.
- Mycorrhizae also produce hormones and antibiotics that enhance root growth and provide disease suppression.
- The fungi benefit by taking nutrients and carbohydrates from the plant roots they live in.

Actinomycetes

- Transitional group between bacteria and fungi
- Active in degrading more resistant organic compounds
- Optimal growth at alkaline pH
- 2 important products: produce antibodies (streptomycin is produced by an actino), produce geosmin
- Negative impact - potato scab (*Streptomyces scabies*)

Algae

- Many different species of algae live in the upper half-inch of the soil.
- Unlike most other soil organisms, algae produce their own food through photosynthesis. It appear as a greenish film on the soil surface following a saturating rain.
- Algae improve soil structure by producing **slimy substances that glue soil together** into water-stable aggregates.
- Some species of algae (the blue-greens) can fix their own nitrogen, some of which is later released to plant roots. Algae are classified as,

- Cyanophyta (Blue-green algae)
- Chlorophyta (Green algae)
- Xanthophyta (Yellow-green algae)
- Bacillariophyta (diatoms or golden-brown algae)
- Blue-green algae and grass-green algae are more abundant in soil.
- The green-grass algae and diatoms are dominant in the soils of temperate region while blue-green algae predominate in tropical soils.
- Green-algae prefer acid soils while blue green algae are commonly found in neutral and alkaline soils.
- The most common genera of green algae found in soil are: *Chlorella, Chlamydomonas*

Protozoa

- Protozoa are free-living microorganisms that crawl or swim in the water between soil particles.
- Many soil protozoa are predatory, eating other microbes.
- One of the most common is an amoeba that eats bacteria. By eating and digesting bacteria, protozoa speed up the cycling of nitrogen from the bacteria, making it more available to plants.

Sr. No.	**QUESTION**	**ANSWER**
1	Which microorganisms is predominant in soil?	Bacteria
2	The region which is adjacent to the root system is called?	Rhizosphere

3	Who coined the term phyllosphere?	Dutch Microbiologist Ruinen
4	The organic material which breackdown is not possible is known as_____.	Humus
5	Which microorganisms is act as predator?	Protozoa
6	Transitional group between bacteria and fungi is known as which microorganisms?	Actinomycetyes

Factors Affecting Distribution, Activity and Population of Soil Microorganisms

Soil microorganisms (Flora & Fauna), just like higher plants depends entirely on soil for their nutrition, growth and activity. The major soil factors which influence the microbial population, distribution and their activity in the soil are

1. Soil fertility
2. Cultural practices
3. Soil moisture
4. Soil temperature
5. Soil aeration
6. Light
7. Soil PH (H-ion Concentration)
8. Organic matter
9. Food and energy supply
10. Nature of soil
11. Microbial associations.

All these factors play a great role in determining not only the number and type of organism but also their activities. Variations in any one or more of these factors may lead to the changes in the activity of the organisms which ultimately affect the soil fertility level. Brief account of all these factors influencing soil micro flora / organisms and their activities is activities are discussed paragraphs.

1. Cultural practices (Tillage):

- Cultural practices viz. cultivation, crop rotation, application of manures and fertilizers, liming and gypsum application, pesticide/fungicide and weedicide application have their effect on soil organism.
- Ploughing and tillage operations facilitate aeration in soil and exposure of soil to sunshine and thereby increase the biological activity of organisms, particularly of bacteria. Crop rotation with legume maintains the favorable microbial population balance, particularly of N2 fixing bacteria and thereby improve soil fertility.
- Liming of acid soils increases activity of bacteria and actinomycetes and lowers the fungal population. Fertilizers and manures applied to the soil for increased crop production, supply food and nutrition not only to the crops but also to microorganisms in soil and thereby proliferate the activity of microbes.
- Foliar or soil application of different chemicals (pesticides, fungicides, nematicides etc.) in agriculture are either degraded by the soil organisms or are liable to leave toxic residues in soil which are hazardous to cause profound reduction in the normal microbial activity in the soil.

2. Soil fertility:

- Fertility level of the soil has a great influence on the microbial population and their activity in soil. The availability of N, P and K required for plants as well as microbes in soil determines the fertility level of soil. On the other hand, soil micro flora has greater influence on the soil fertility level.

3. Soil moisture:

- It is one of the important factors influencing the microbial population & their activity in soil.
- Water (soil moisture) is useful to the microorganisms in two ways i.e., it serves as source of nutrients and supplies hydrogen / oxygen to the organisms and it serve as solvent and carrier of other food nutrients to the microorganisms.
- Microbial activity & population proliferate best in the moisture range of 20% to 60%.
- Under excess moisture conditions / water logged conditions due to lack of soil aeration (Oxygen) anaerobic microflora become active and the aerobes get suppressed.
- While in the absence of adequate moisture in soil, some of microbes die out due to tissue dehydration and some of them change their forms into resting stages spores or cysts and tide over adverse conditions.
- Therefore, optimum soil moisture (range 20 to 60 %) must be there for better population and activity of microbes in soil.

4. Soil temperature:

- Next to moisture, temperature is the most important environmental factor influencing the

biological physical & chemical processes and of microbes, microbial activity and population in soil.

- Though microorganisms can tolerate extreme temperature (such as - 60 ° or + 60 u) conditions, but the optimum temperature range at which soil microorganisms can grow and function actively is rather narrow.

- Depending upon the temperature range at which microorganisms can grow and function, are divided into three groups i.e., psychrophiles (growing at low temperature below 10 °C) Mesophiles (growing well in the temp range of 20 ° C to 45° C) and thermopiles (can tolerate temperature above 45° C and optimum 45-60°C).

- Most of the soil microorganisms are mesophilic (25 to 40 °) and optimum temperature for most mesophiles is 37° C.

- True psychrophiles are almost absent in soil, and thermopiles though present in soil behaves like mesophiles. True thermopiles are more abundant in decaying manure and compost heaps where high temperature prevails.

- Seasonal changes in soil temperature affect microbial population and their activity especially in temperate regions. In winter, when temperature is low (below 50° C), the number and activity of microorganisms falls down, and as the soils warms up in spring, they increase in number as well as activity. In general, population and activities of soil microorganisms are the highest in spring and lowest in winter season.

1. **Light:** Direct sunlight is highly injurious to most of the microorganisms except algae. Therefore, upper portion of the surface soil a centimeter or less is usually sterile or devoid of microorganisms. Effect of sunlight is due to heating and increase in temperature (More than 45°)

2. **Soil Reaction / Soil PH:** Soil reaction has a definite influence / effect on quantitative and qualitative composite on of soil microbes. Most of the soil bacteria, blue-green algae, diatoms and protozoa prefer a neutral or slightly alkaline reaction between PH 4.5 and 8.0 and fungi grow in acidic reaction between PH 4.5 and 6.5 while actinomycetes prefer slightly alkaline soil reactions. Soil reactions also influence the type of the bacteria present in soil. For example, nitrifying bacteria *(Nitrosomonas & Nitrobacter)* and diazotrophs like *Azotobacter* are absent totally or inactive in acid soils, while diazotrophs like *Beijerinckia, Derxia,* and sulphur oxidizing bacteria like *Thiobacillus thiooxidans* are active in acidic soils.

3. **Soil Organic Matter:** The organic matter in soil being the chief source of energy and food for most of the soil organisms, it has great influence on the microbial population. Organic matter influence directly or indirectly on the population and activity of soil microorganisms. It influences the structure and texture of soil and thereby activity of the microorganisms.

4. **Soil air (Aeration):** For the growth of microorganism better aeration (oxygen and sometimes CO2) in the soil is essential. Microbes consume oxygen from soil air and gives out carbon dioxide. Activities of soil microbes is often measured in terms of the amount of oxygen absorbed or amount of Co2 evolved by the organisms in the soil environment. Under high soil moisture level / water logged conditions, gaseous exchange is hindered and the accumulation of Co4 occurs in soil air which is toxic to microbes. Depending upon oxygen requirements, soil microorganisms are grouped into

categories viz aerobic (require oxygen for like processes), anaerobic (do not require oxygen) and microaerophilic (requiring low concentration / level of oxygen).

5. **Food and energy supply:** Almost all microorganisms obtain their food and energy from the plant residues or organic matter / substances added to the soil. Energy is required for the metabolic activities of microorganisms. The heterotrophs utilize the energy liberated during the oxidation of complex organic compounds in soil, while autotrophs meet their energy requirement form oxidation of simple inorganic compounds (chemoautotroph) or from solar radiation (Photoautotroph). Thus, the source of food and energy rich material is essential for the microbial activity in soil. The organic matter, therefore serves both as a source of food nutrients as well as energy required by the soil organisms.

6. **Nature of Soil:** The physical, chemical and physio-chemical nature of soil and its nutrient status influence the microbial population both quantitatively and qualitatively. The chemical nature of soil has considerable effect on microbial population in soil. The soils in good physical condition have better aeration and moisture content which is essential for optimum microbial activity. Similarly, nutrients (macro and micro) and organic constituents of humus are responsible for absence or presence of certain type of microorganisms and their activity. For example, activity and presence of nitrogen fixing bacteria is greatly influenced by the availability of molybdenum and absence of available phosphate restricts the growth of *Azotobacter*.

7. **Microbial associations / interactions:** Microorganisms interact with each other giving rise to antagonistic or symbiotic interactions. The association existing between one organism and another whether of symbiotic or antagonistic influences the population and activity of soil microbes to a great extent. The predatory habit of protozoa and some mycobacteria which feed on bacteria may suppress or eliminate certain bacteria. On the other hand, the activities of some of the microorganisms are beneficial to each other. For instance, organic acids liberated by fungi, increase in oxygen by the activity of algae, change in soil reaction etc. favors the activity or bacteria and other organisms in soil.

8. **Root Exudates:** In the soil where plants are growing the root exudates also affects the distribution, density and activity of soil microorganism. Root exudates and sloughed off material of root surfaces provide an abundant source of energy and nutrients and thus directly or indirectly influence the quality as well as quantity of microorganisms in the rhizosphere region. Root exudates contain sugars, organic acids, amino acids, sterols, vitamins and other growth factors which have the profound effecton soil microbes.

Sr. no.	QUESTION	ANSWER
1	How many factors affect microbial activity of soil?	Eleven
2	Which nutrient is used for check microbial activity?	N, P, and K
3	Optimum soil moisture content is ____?	20-30%
4	requiring low concentration / level of oxygen is known as ?	Microaerophilic microorganism

5	Name of microorganism which associate asymbiotically with roots.	*Azotobacter*
6	pH range required for growth of fungi is _____.	4.5-8 pH

NITROGEN CYCLE

Introduction:

- Nitrogen is an important element to all life processes on Earth. To appreciate the importance of nitrogen in our biosphere, simply realize nitrogen comprises 78% of the atmosphere, and is embedded in every living tissue.
- It is a component of amino acids, proteins and nucleic acids. With the exception of carbon, nitrogen is the most universal element of life Put simply: Life could not exist without nitrogen.
- Nitrogenous compounds are also required by some organisms for metabolic functions and respiration.
- The nitrogen cycle is defined as the sequence of change from free atmospheric nitrogen to fixed inorganic nitrogen, to simple organic compounds, to complex organic compounds in the tissue of plants, animal and microbes and the release of this nitrogen back to atmospheric nitrogen.

Proteolysis:

- The nitrogen in protein is locked and is not available as a nutrient for plants. This organically bound nitrogen became free for reuse by the process of enzymatic hydrolysis of protein that is called proteolysis.
- Protein is converted into the small units (peptides) by extracellular proteinases enzyme produced by some microbes.
- These peptides are then attacked by peptidases and ultimately individual amino acids are released. The overall reaction may be summarized

Proteins $\xrightarrow{\text{Protease}}$ Peptides $\xrightarrow{\text{Peptidase}}$ Amino acids

Ammonification:

- The end product of proteolysis is amino acids. They are utilized as a nutrient by microbes or degraded by microbial attack.
- The process in which liberation of nitrogen or removal of amino group from the amino acid is called as deamination. There are some variation of domination reactions are also exhibit by microbes, one of the end product is always ammonia.

Alanine deamination

$CH_3CHNH_2COOH + ½ O_2 \longrightarrow CH_3COCOOH + NH_3$

Alanine — Pyruvic acid — Ammonia

- The production of ammonia is referred as ammonification. Ammonia is volatile and if it is solubilized it leave the soil and NH_4^+ is formed. These ammonium ions may be accumulated and utilized by plants and microorganisms and under favorable condition it get oxidized to nitrates.

Nitrification:

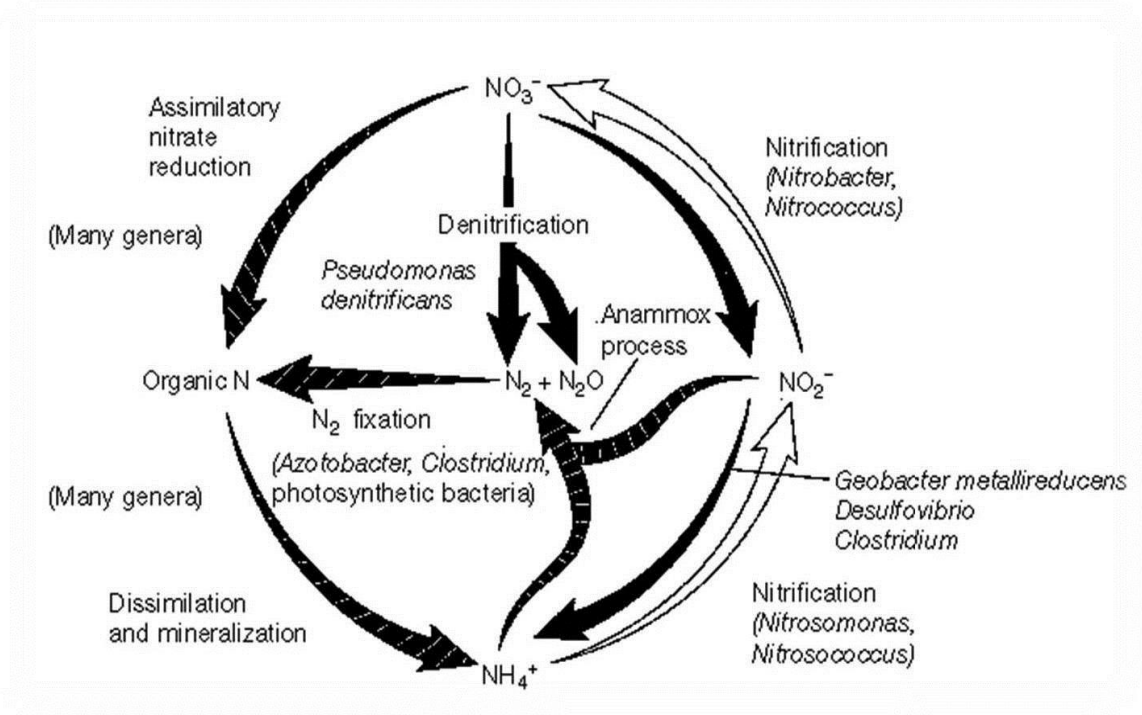
- Microorganisms convert ammonia to nitrate and this process is called nitrification.
- This process occurs in two steps. Each step performed by a different group of bacteria.
- Oxidation of ammonia to nitrite by ammonia oxidizing bacteria.

$2NH_3 + 3O_2 \longrightarrow 2HNO_2 + 2H_2O$

- Oxidation of nitrite to nitrate by nitrite oxidizing bacteria.

$HNO_2 + ½O_2 \longrightarrow HNO_3$

- These both groups of bacteria, ammonia oxidizer and nitrite oxidizer, are Gram negative chemolithotrophs. Nitrifying bacteria occur widely in variety of habitats, including soil, sewage and aquatic environments.
- The following species are recognized as ammonia oxidizers:
  - *Nitrosomonas europaea,*
  - *Nitrosococcus oceanus,*
  - *Nitrosovibrio tenuis,*
  - *Nitrosococcus nitrosus*
- And only a few species are recognized as nitrite oxidizer:
  - *Nitrobacter winogradskyi,*
  - *Nitrospina gracilis*
- Nitrification process was discovered by Schloesing and Muntz in 1877 and the bacteria responsible for that were isolated by Winogradskyi in 1890.



Nitrate reduction:

- Several heterotrophic bacteria are capable of converting nitrates into nitrites or ammonia.
- This process generally occurs under anaerobic conditions. the oxygen of the nitrate serves as an electron acceptor for electrons and hydrogen. This process involves several reactions and they are summarized as below:

$$HNO_3 + 4H_2 \longrightarrow NH_3 + 3H_2O_2$$

Denitrification:

- The transformation of nitrates to gaseous nitrogen by the microorganisms in a series of biochemical reaction. This process is known as gentrification. This process is undesirable in the agricultural point of view because here the loss of the nitrogen occurs from the soil.
- There are several species of bacteria are capable to convert NO_3^- to N_2:

E.g., *Achromobacter,*
Agrobacterium,
Alkaligens,
Bacillus,
Pseudomonas,
Thiobacillus,
Flavobacterium,
Vibrio

The overall biochemical reaction is summarized as below:

$2NO_3^- \longrightarrow 2NO_2^- \longrightarrow 2NO \longrightarrow N_2O \longrightarrow N_2$

Nitrate nitrite nitric oxide nitrous oxide nitrogen

Nitrogen fixation:

This process can be carried out by aerobic or anaerobic prokaryotes and does not occur in eukaryotes.

- Under aerobic conditions a wide range of free-living microbial genera (*Azotobacter, Azospirillum*) contribute to this process.
- Under anaerobic conditions the most important free-living nitrogen fixers are members of the genus *Clostridium*. Nitrogen fixation by *cyanobacteria* such as *Anabaena* and *Oscillatoria* can lead to the enrichment of aquatic environments with nitrogen.
- In addition, nitrogen fixation can occur through the activities of bacteria that develop symbiotic associations with plants.
- The nitrogen-fixation process involves a sequence of reduction steps that require major energy expenditures the essential reactants in the bacterial nitrogen fixation process as follow:

1) nitrogenase enzyme complex

2) nitrogenase reductase

3) Strong reducing agents such as ferredoxin and flavoprotein

4) ATP

5) A regulating system for NH3 production and utilization

6) A system that protects the nitrogen fixing system from inhibition by molecular oxygen.

Sr. No	QUESTION	ANSWER
1	The example of symbiotic nitrogen fixer is____.	*Rhizobium*
2	How many steps of nitrogen cycle?	Five
3	Protein converted in to peptides by which enzymes?	Protease
4	Nitrogen fixation does not occur in which organisms?	Eukaryotes
5	Microorganisms convert ammonia to nitrate and this process is called_____.	Nitrification

6	Which species are recognized as nitrite oxidizer?	*Nitrobacter winogradskyi, Nitrospina gracilis*

SULFUR CYCLE

Sulfur reduction and oxidation and Winogradsky Column:

Introduction:

- Sulfur, like nitrogen and carbon passes through a cycle of transformation mediated by microorganisms.
- Some species oxidize and some other reduces various sulfur compounds.
- Such biogeochemical cycles are important in geology because they affect many minerals. Biogeochemical cycles of sulfur are also important for life because sulfur is an essential element, being a constituent of many proteins and cofactors.
- There are some sequences of events involved in this cycle may be summarized as follows: Photosynthetic and chemosynthetic microorganisms contribute to the environmental sulfur cycle.

1) The elemental sulfur (s) cannot be utilized by plants and animals. Certain bacteria are capable to oxidize sulfur to sulphates. This reaction is carried out by *Thiobacillus thioxidans*, an autotroph bacterium.

The reaction involved is

$$2S + 2H_2O + 3O_2 \longrightarrow 2H_2SO_4$$

2) Now sulphate is utilized by plants and is incorporated into sulfur containing amino acid and into protein. During the proteolysis process, amino acid liberates, in this some contain sulfur (Sulfur contain amino acid). By the enzymatic activity of many heterotrophic bacteria, this sulfur is released from the amino acid.

For e.g.,

$$\text{Cysteine} + H_2O \longrightarrow \text{pyruvic acid} + H_2S$$

3) Sulphate may reduce to hydrogen sulfide (H_2S) by soil microbes. An example of bacteria involved in this reaction is the genus Desulfotomaculum.

The reaction is:

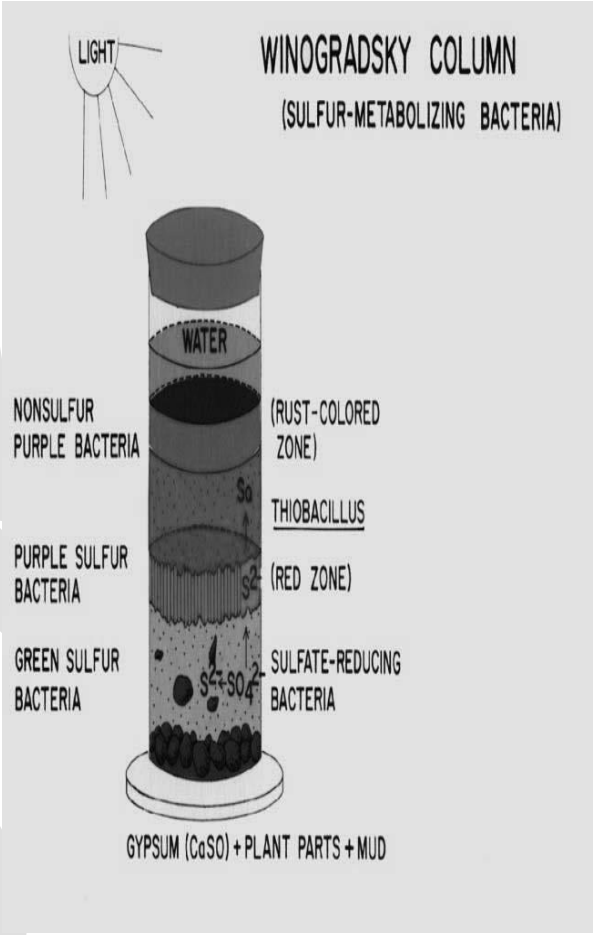
$$4H_2 + CaSO_4 \longrightarrow H_2S + Ca(OH)_2 + 2H_2O$$

4) This H_2S is oxidized to elemental sulfur. This reaction is followed by certain pigmented or photosynthetic sulfur bacteria and is expressed as:

$$CO_2 + 2H_2S \longrightarrow (CH_2O)X + H_2O + 2S$$

Carbohydrate

Winogradsky's Column:



- Sergei Winogradsky was among the first microbiologists to investigate the organisms found in complex biofilm communities. Winogradsky isolated organisms from nature was a miniature model pond cross section which has since been called a Winogradsky column.
- This column contains mud, CaSO4, plant tissue, and water.
- The column is constructed of soil or mud from any source and waterfrom the same or a different source.
- To these natural components, are added supplemental carbon and sulfur.
- Above the soil is a layer of water and the column is usually covered to retard evaporation. The entire column is then illuminated to encourage the growth of phototrophs.
- Then it is exposed to day light and incubated at room temperature. The resulting growth of microorganisms can be quite spectacular and colorful.
- The following microbiological events are occurring into the column.

1) A variety of heterotrophic organisms oxidize various substrates. these organisms decrease the level of O_2 into the column and create an anaerobic condition.

$$\text{Organic matter} + O_2 \longrightarrow \text{organic residue} + CO_2$$

2) Organic acid serves as the electron donors for the reduction of sulfates and sulfites to hydrogen sulfide by anaerobic sulfatereducing bacteria, for e.g., *Desulfotomaculum:*

$$\text{Organic acid} + SO_4^{2-} \longrightarrow H_2S + CO_2$$

3) Photosynthetic microbes like purple and green sulfur bacteria use H_2S as electron donor to reduce CO_2 Sunlight

$$CO_2 + H_2S \longrightarrow (CH_2O)_x + S$$

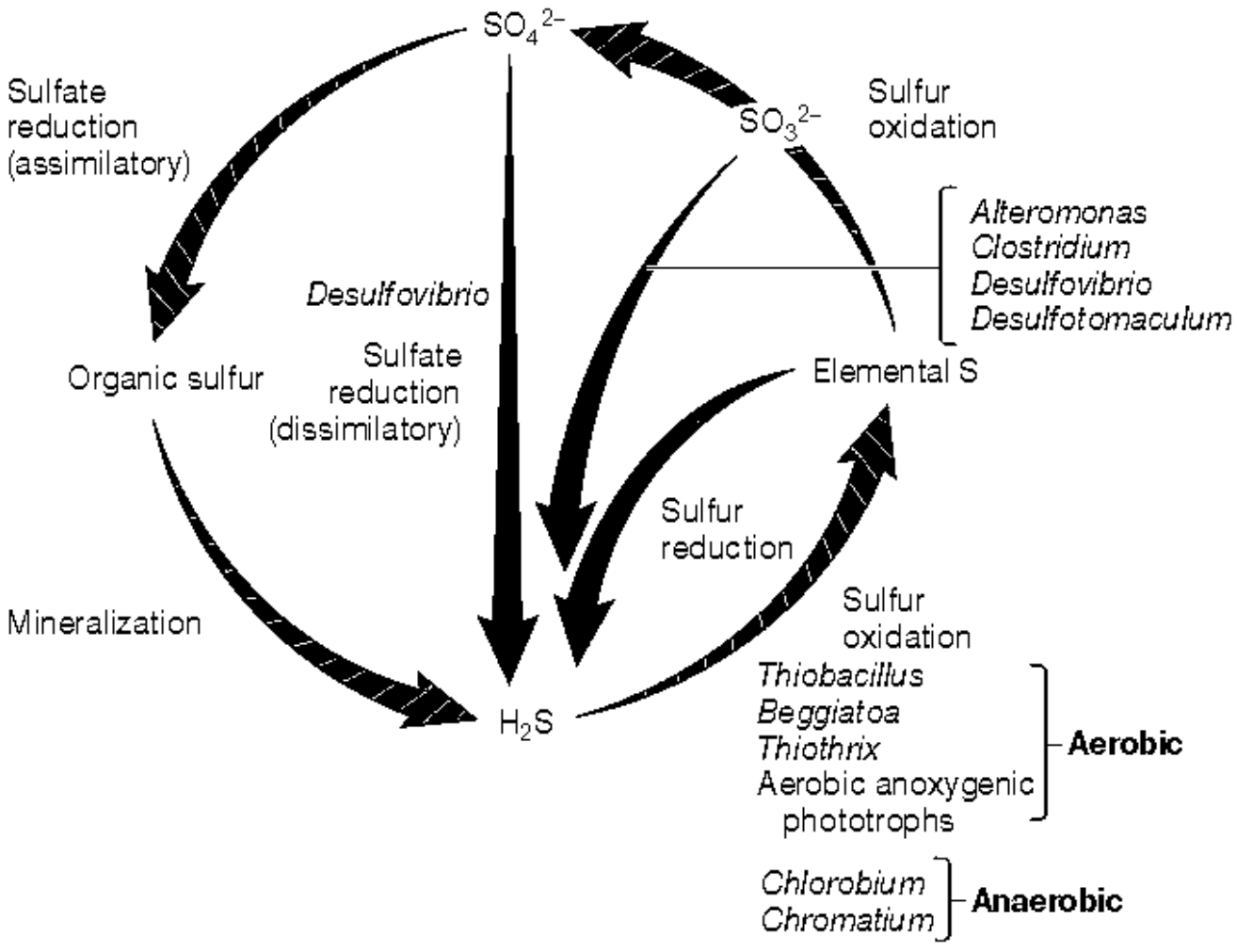
4) The aerobic sulfur metabolizing bacteria for e.g., *Thiobacillus spp*. Develop in the upper portion of the column and it can oxidize reduced sulfur compounds that is SO_4^{2-}, S°, SO_3^-

Reduced sulfur compounds $\longrightarrow$ SO_4^{2-} + accumulation of S

5) The non-sulfur purple bacteria for e.g. *Rhodospirrillum, Rhodomicrobium* are facultative phototrophs and are capable to convert hydrogen gas as an electron donor in photosynthesis.

$$CO_2 + H_2S \xrightarrow{\text{Light}} (CH_2O)_x + S$$

$$CO_2 + 2H_2 \xrightarrow{\text{Light}} (CH_2O)_x + H_2O$$



Sr.no.	QUESTION	ANSWER
1	Who is the father of soil microbiology?	Sergie winogradwsky
2	Winogradsky column is a _____ column.	Sulfur
3	How many types of bacteria present in column?	Three
4	Which types of bacteria present in column?	Purple sulfur, purple non sulfur and green sulfur bacteria
5	Give an example of anaerobic sulfate reducing bacteria.	*Desulfotomaculum*

Carbon cycle

Introduction:

Carbon is the most important element in the biological world. Plants and microbial cell contain large amount of carbon approx. 40 to 50 percent on the dry weight basis. This huge amount of carbon comes into biological world from atmospheric carbon dioxide.

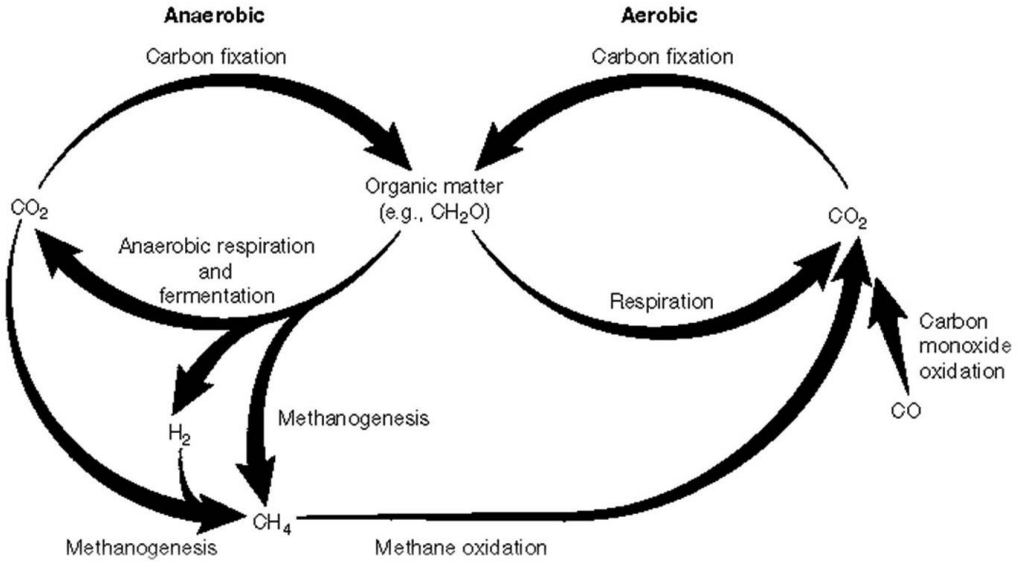


Fig.: The Basic Carbon Cycle in the Environment.

Carbon fixation can occur through the activities of photoautotrophic and chemoautotrophic microorganisms.

Carbon dioxide fixation:

- The atmospheric nitrogen is fixed into the soil through the carbon fixation process by photoautotroph that is green plants and algae. These photoautotrophs supply the organic nutrients in the form of complex carbon compounds which is needed for heterotrophic animals and chlorophyll lacking microbes.
- Once the atmospheric carbon is fixed by photoautotrophs, in presence of light it became no available for the generation of new plant life.
- Therefore, it is essential to carbonaceous material to be decomposed and returned to the environment.

Degradation of complex organic compound:

- The organic carbon compounds that are deposited in the soil are degraded by microbial activity. The end product CO_2 is released into the air and soil.
- The most abundant material in plants is cellulose. It is attacked by many species of bacteria and fungi.
- The initial attack is by cellulase enzyme which split the long chain polymer of glucose to cellobiose.

  Cellulose → cellobiose
- Then cellobiose splits to glucose by the enzyme system of many microbes.

  Cellobiose → glucose

  Glucose → CO2, water & other end products
- Similar degradation pathway occurs for the other major plant tissue substance such as hemicelluloses, lignin and pectin. Carbon dioxide may also originate from the decarboxylation of amino acid and from the dissimilation of fatty acids. All these events occur in soil.

Humus formation and its important:

- It is a complex organic matter derived from decomposition of plants and animal tissue by the action of various soil microbes. Humus is a brownish black material of soil and is of great importance for plant growth. it improves the physical condition of soil like bettering texture and water holding capacity and forming reservoir of mineral nutrients.
- Chemically humus is composed of dead organic matter alone, but sometimes various types of salts may be associated with it.

- That it's a source of nutrients and that it improves soil structure are two reasons why humus is important. Humus is dark brown, fresh smelling, and nutrient rich organic matter. That it contains such important elements as nitrogen makes it a source of nutrients for plant roots, soil, and soil food web members.

- That it encourages the formation of air and water pore spaces makes it a facilitator of soil structure. The soil that has adequate air and water pore space has appropriate moisture levels and adequate drainage. Both of these contributions from the presence of humus in soil account for the improved well- being of soil, soil food web critters, and plant parts above and below ground surface levels.

- Humus helps the soil retain moisture and encourages the formation of a good soil structure. They also bind the plant nutrients, making them more available.

- The dark color of humus (usually black or dark brown) helps to warm up cold soils in the spring.

- During the humification process, microbes secrete sticky gum-like mucilage; these contribute to the crumb structure of the soil by holding particles together, and allowing greater aeration of the soil.

Sr. no	QUESTION	ANSWER
1	Color of humus is____.	Dark brown
2	During humification process microbes secrete which substance?	Sticky gum like subatance
3	How many steps of carbon cycle?	Three
4	What is the first step in carbon cycle?	Carbon dioxide fixation
5	Cellulose is converted into which final product which is easily utilized by microorganisms?	Glucose

Interaction within, between & among populations Introduction:

- Members of Biotic communities in an area are dependent on one another. The inter dependences is reflected in their interactions mainly for Food, Space, Reproduction, Protection.

- These interactions are important for survival of different species & communities as a group.

- Interaction means to come in contact when one population of species come in contact with other population of species in a community are known as species interactions.

- Species interactions are broadly categorized in to following interactions or associations:

1. Neutral interactions

2. Positive interactions (Beneficial)
3. Negative interactions (Inhibition)

Neutral Association:

- When none of the species affect each other in any way, then this interaction are known as "neutral interactions".

Neutralism

- When different species occupy in the environment without affecting each other than these phenomena is called neutralism.
- **E.g.,** Here different species of microbes present in soil, Organism utilize different nutrients & produce end products not inhibiting other species.

Positive Interactions:

- Some of the interactions between species in the community benefits one or both the species is known as "positive interaction".
- There are two types of positive interactions

1. Mutualism
2. Commensalism

Mutualism

- An association of two species in which both species are benefited is called mutualism.
- Mutualism may or may not involve close physical association between the individual of pairs of species.
- The condition in which there is a close physical association between the individuals of pairs of species is called "symbiosis".
- There is two kind of mutualism:-

a) Obligate mutualism

- When species are completely dependent upon each other is known as "obligate mutualism".

E.g., Rhizobium:

- These are nitrogen fixing bacteria living in root nodules of legumes, where the bacteria deriving nutrition from the host plant & fixing nitrogen, which is available to the plants.

E.g., Lichen:

- It is example of syntrophism.
- Exchange of nutrients between two species is called "syntrophism". Syn- together, Trophism-feeding
- Algae and fungi exhibit mutually beneficial relationship in "Lichens".
- It has a top layer of fungus middle layer of algae & bottom layer of fungus.
- Bottom layer holds the structure tightly & firmly to the substance like rock, wood etc.….

E.g., Mycorrhizae:

- It is fungus lives in rhizosphere.
- It gives mutualistic relationship between fungi and roots of about 80% of higher plants.
- Fungus helps in mineral nutrition of the plants from soil & then carbohydrates derive by fungus from plants.

E.g., Gut flora:

- Bacteria present in the gut of some animals helps in cellulose digestion while bacteria get nutrients, space and protection against adverse condition.

b) Facultative Mutualism:

- "When one species may survive even in the absence of the other partner species is called facultative mutualism."

E.g., Sea anemone:

- It gets attachment with the shell of hermit crab.
- Sea anemone grows on the back of crab providing camouflage & protection and also it is transported for reaching new food sources.
- This type of mutualism is also called "Protocooperation."
- Protocooperation: - When one species derive protection with cooperation of another species.

Commensalism

- When a relationship between two species occur where one species is benefited while other species neither gets any benefit nor is adversely affected under normal condition this interaction is known as "commensalism".
- Some organisms live inside the bodies of large animals in order to protect themselves from enemies and adverse condition.

E.g., Sucker fish & Shark

- The sucker fish attaches to shake surface with the help of its dorsal fin. Sucker fish is dispersed to distance areas with better food supply.
- The fish get protection from predators due to its association with shark.
- However, the shark doesn't get any benefit from sucker fish & it is also not affected adversely.

E.g., Epiphytes

- Epiphytes like mosses, ferns and orchids growing on trees benefit from better light condition but they do not harm the trees.
- Many fungi degrade cellulose to glucose & organic acids which helps many bacteria to grow when they are unable to affect cellulose but they do not harm the fungi.

Negative Interaction:

- When certain interaction between different species adversely effects on either one or both species is known as "negative interaction" or "negative association".
- Here, the association or interaction results in the negative effects on different species. Mainly there are four types of negative interactions.

1. Competition
2. Predation
3. Parasitism
4. Antagonism

Competition

- When interaction between two species occurs where both species suffer adverse effects or harmfully kill is known as competition.
- Usually, competition occurs when resources such as space, light, and nutrients etc. are in short supply which result of competition.
- Competition reduces the growth and speed of reproduction of both species. Competition is basically of two types:

Inter specific competition

- Interactions occurs between individuals of two different species occurring in a habitat It is less harmful.

Intra specific competition

- Interactions occur between individuals of the same species.
- The requirements of individuals of the same species are very similar so that they compete more fiercely.

Predation

- Interaction between species involving killing & consumption of prey is called predation.
- The species which eats the other is called predator and one consumed by predator is called prey. Predation is commonly illustrated by the Herbivore-Carnivore interaction (Grass-Deer- Tiger Food chain).

Parasitism

- When the species are smaller in size (The parasite) and live on or in the large species from which it obtains food this interaction is called "parasitism".
- Plants like cuscuta, Rafflesia are parasitic plants which live on other flowering plants.
  There are two types of parasites which remain outside or inside the host.

Ecto parasites

- They attach to the skin & hair of the host some of them use suckers, clamps, adhesive surface and sucking mouth.

Endo parasites

- Parasites remain inside the host & cause disease in host is called Endo parasites. Bacteria, virus etc.

Antagonism

- When one species of organisms are inhibited by another species by producing growth inhibiting substance (antibiotics) is known as "antagonism".

Sr. No.	QUESTION	ANSWER
1	How many types of interaction are there between microorganisms?	Three
2	Which are the interactions?	Beneficial, harmful and neutral
3	Symbiotic association is also known as_____.	Mutualisms
4	Interaction between species involving killing & consumption of prey is called _____.	Predation

5	Parasites remain inside the host & cause disease in host is called _____.	Endo parasites
6	Give an example of ecto parasite.	suckers, clamps
7	How many types of negative interaction?	Four