Water

Water considered to be one of the most important components of the environment. which play vital role to the existence of all living organisms, but this valued resource is increasingly being in danger as human populations grow and demand more water of high quality for domestic purposes and economic activities, so contamination of water is now a major problem in the global world as a consequence of industrialization, population growth and urbanization.

Water quality parameter

1-Temperature:

The most important source of heat for fresh water is generally the sun also can be affected by the temperature of changes in air and ground temperatures. So changes in water temperature are iinked to changes in water quality.

2-pH:

pH is a measurement of the hydrogen ion (H') concentration in water, and is usually used to describe the acid/base balance of water.

3-Turbidity:

Turbidity is a measure of the water's lacks of clarity. Many factors contributing in Highly turbid water such as Particulates and these include silt, clay, organic matter, algae and other microorganisms and any other pariculaie maiter.

4-Total Dissolved Solid (TDS):

Total dissolved solids (TDS) represent a mcasurc of the amount of dissolved material in water.

5-Dissolved Oxygen (DO):

Dissolved oxygen (DO) is very important parameter and essential for water quality, ecological status, health of a river and assessing the suitability of water for drinking all this because of its importance as a respiratory gas, and its use in biological and chemical reactions.

Lab 1-2

Ways and means to collect water samples for the purpose of studies of microbiological Water makes up about 71% of the of the earth's surface, water is the middle of an environmental very large and diverse, So when you ta water samples for the purposes of the studies microbiological taking into consideration very important points as you must know son must information about those waters such as the nature of these bodies of water (oceans, seas, rivers, lakes, ponds, Sewage and agricultural water ete) and also find information about the depths to be studied and Information on the chemical and physical properties land vital) to those waters as knowledge dissolved oxygen content, temperature, the proportion of soluble salts and hydrogen ion concentration and water content of organic matter and the degree of turbidity.

Some points taking into consideration of water samples to be examination of microbial:

1. Must be taking the sample of the area to be studied in terms of location and depth, where the recorded observations about the combination being a site near a residential area or near factories or hospitals.

2. Must be use the correct statistical methods take into account u determining the many samples that are taken for the purpose of study,

3. Water samples are collected in a wide crater bottles with tight lie avoid any kind of contamination.

4. When taking the sample of tap water (drinking water) should be sterilized nozzle tap before taking the sample then leave to cool (This method is used for the purpose of drinking water test) However, if the purpose is to know Sources of pollution don't sterilized bottle nozzle.

5. Water samples must be taken to reverse the trend and careful hands of contact the water sample or any other external thing.

6. Leave a space inside the bottles to allow creating aerobic conditions inside the bottle and at the same time allow the sample shake before testing them.

7. Some material may be added to the water sample, such as sodium sulfate to pull chlorine Cl that deadly microscopic organisms in the water, as well as the added material EDTA chelating substance is working to pull the iron from the water.

Isolation and enumeration of water bacteria

Bacteria present in the water division into several groups:

1. Bacteria that live in nature and mostly located within gram negative bacteria and include many types belonging to the genera.- Acinetobacter, Cytophaga, Flavobacterium, Pseudomonas, Chromobacterium. And there are many gram positive bacteria a few such as Micrococcus, Bacillus.
2. Soil bacteria source and mostly follows the genus Bacillus.
3. Bacteria sourced from human and animal gut as a result of water pollution with human and animal waste such as E. coli coliform bacteria.
4. The bacteria can enter the water through the air or with rainwater such as Streptomyces, Bacillus.

Bacteria

There are groups of bacteria that grow in water, which indicator to contamination

1- The first group of bacteria that grow of (20-22), transmitted water by soil, air and plants which refers to existence of non-purity water.

2- The second group which grow of (37%) and cause disease human and animal, transmitted to water by sewage, human and animal water.

3- Bacteria that polluted water by wastes: include important types of Coliform, Clostridium, and Lactobacillus.

Total coliform group (TC): include different types of bacteria that line colon.

1-Fecal Coliform (FC): include E. coli that her source human wastes.

2- Fecal Streptococcus (FS): that presence show on wastes contamination.

FC: found in human wastes more than animals wastes on contrary of (FS).
FS>**FC:** contamination source in water is animals wastes, and true contrary.

$\frac{FC}{FS} > 4.4$: Contamination source in water is sewage which found of human wastes.

$\frac{FC}{FS} > 0.7$: contamination source in water is animals wastes.

$4.4 - 0.7$: contamination source in water are human and animals wastes

Detection and diagnosis of coliform as an indicator to water pollution instead of other bacteria for several reasons:

1- Their presence in large numbers
2- Live of longer period compared with disease and intestinal germs know long term and recent pollution of wastes.

There are many ways used to account bacteria in the water and the following is a review of some of them:

The total bacterial count in water:

A. Plate count technique method

Materials and method:

1. Take water samples from various sources such as river water, tap water.
2. Prepare dilution series $10^{-1}, 10^{-2}, 10^{-3}\ and\ 10^{-4}$ (work three replicates for each dilution).
3. Put Iml from each dilution in a petri dish.
4. Pour the dissolved middle nutritious Casein Peptone Starch (CPS) at degree 45 C and mixed well with the dilution sample and placed and leave solidifies.
5. Incubate the first set of dishes at 25C degree for 14-7 days; the second group incubate at degree 37C for two days.
6. After the expiration of the incubate count colonies in the dishes were a number of colonies between 300-30 colony.
7. Calculating the number of bacterial cells per Iml of water following application:

$$\boldsymbol{The\ number\ of\ cells\ in\ (lcm3)\ 1ml = number\ of\ colonies\ \times\ inverted\ dilution}$$

B. Most probable number method (M.P.N)

1-Different water models such as river water, sea, Sewage water taken, $100\ ml$ size.

2-Prepare three groups of test tubes (each set of five test tubes) Put in the first group 5 ml of double concentration of liquid casein peptone starch (C.P.S). And put in each test tube of the second set $9\ ml$ and the third 9.9 ml of (C.P.S) normal concentration, then the sterility the three groups.

Inoculate all test tube of the first set with double concentration with 5 ml of water sample. Then inoculated each test tube of the second set with Iml of water sample, Finally Inoculate each test tube of third group with 0.1 ml of water sample.

3-Shake the test tubes quietly to ensure homogeneity.

4- Incubate the tubes in 37c for 24-48 hr.

5-Check the tubes that give a positive test (How?)

Layer composition, turbidity, sediment composition and viscosity increase.

6- Use the attached table to calculate the number of bacterial cells in 100 ml of water.

Drinking water classification in some European countries

The total number of bacteria in 1 ml	Class drinking water
Less than 10	Complete purity water Purify water
10-100	Very purify water
100 – 1000	purify water
1000 -10000	Medium purify water
10000- 100000	Contaminated water
More than 100000	Very contaminated water

Soil microbes

Soil is an interesting medium for growing microorganisms. It contains various nutrients that the microbes need for their metabolism. Unfortunately, the nutrients are not always readily available. Soil is not a homogeneous material) but rather is a heterogeneous material. Soil consists of round rock particles, Clay particles, and silt particles in various combinations and with varying amounts of water. The solid particles form layers or clumps with open void spaces between the different soil particles. Microorganisms cannot penetrate the solid soil particles. They can live on the surface of the particles, provided there are adequate nutrients and water for their growth. Organic matter discharged from living organisms on the soil surface provides the major nutrients for the microorganisms in the soil. The upper layer of soil provides the best environment for growing microorganisms. Bacteria, fungi, actinomycetes, algae, protozoa and nematodes can be found in organic rich soil. The soil environment determines the specific microbes that can grow and their numbers.

Microorganisms

Moisture, nutrients and particulate surfaces are essential for the growth of microorganisms in the soil. The gaseous atmosphere in the void spaces is also important in microbial growth. The majority of microorganisms in soil are aerobic, requiring unsaturated conditions.' Only anaerobic organisms grow in soils saturated with water.

Bacteria

The diversity of nutrients found in the soil stimulates bacteria that can completely metabolize the organics to carbon dioxide, Pseudomonas, Bacillus, Arthrobacter, Clostridium, Achromobacler, Micrococcus and Flavobacterium as the most common bacteria in organic soils. Nitrifying bacteria are also widely distributed in soils

Fungi

The fungi find the upper layers of soil favorable for growth. As strict aerobes, the fungi are only able to grow when there is adequate oxygen. The large quantity of plant residues deposited on the soil surface provides the nutrients needed by certain species of fungi. Lignin is a complex aromatic polymer that combines with cellulose in higher plant tissue to protect the plant tissue from premature biodegradation.

A number of fungi have the ability to metabolize the lignin and the cellulose in dead plant tissue, returning these materials back into the environment as basic components. Yeasts are a special group of fungi that will also be found in soils, the number of fungi cells range from 10/g soil to 10%g soil. There is no doubt that fungi are important to the overall soil microbiology.

Actinomycetes

Ctinomycetes will also be found in soil. As previously indicated, the Actinomycetes are bacteria that have some characteristics of bacteria and some characteristics of fungi.

Algae

Algae are the most common photosynthetic microorganisms found in soil. They will be found only near the soil surfaces where light is readily available.

Protozoa

Single cell animals find the bacteria in soil a good source of nutrients. It is not surprising that protozoa are found in the upper layers of the soil where there is adequate moisture, oxygen, and bacteria. Sarcodina have the ability to crawl over the soil surfaces and engulf the bacteria attached to the soil particles. Mastigophora and Ciliata will also be found in soils. The small flagellated protozoa are able to find sufficient nutrients to grow nicely. Small free-swimming ciliated protozoa are also found where the bacteria populations are very active. It takes more energy for the ciliated protozoa to survive than the other protozoa. When the environment becomes unsuitable for protozoa growth, they produce cysts that allow the cells to survive until the environment becomes suitable again. The number of protozoa cells range from 10^3 to 10^5 protozoa/g soil.

Higher animals

Higher animals also find soil a reasonable place for living. Various worms and mites can be found in the soil. Nematodes and other round worms are found in soil. Earthworms are important aerators for surface soils, creating increased void spaces.

So we can conclude some characteristics about soil microbes.

1-Bacteria, fungi, actinomycetes, yeast, protozoa and nematodes can be found in the upper layers of soil.

2- Environmental conditions in the soil determine the distribution of microbes and their survival in soil.

3-Microbial activity in soils is a factor in determining the chemical quality of groundwater.

4- Microbes exist in soil capable of metabolizing all naturally occurring organic materials and related synthetic compounds.

5- Contaminated soils arc a good source of bacteria capable of metabolizing the contaminating organics.

6- Bacteria occur in deep soils, but lack the ability to metabolize and grow in that environment.

Lab 3

Collection of soil sample

1. Taking in consideration type of soil fertilized or not fertilized soil, salty or not.
2. Remove the superficial layer of the soil because it's contaminated by so many environmental factors result from human activities, exposure to the U.V light.
3. The sample should be taken from 3-5 cm under the surface of the soil.
4. Take several samples (at least 5) from each site and these sample must collected randomly.
5. Clean the sample from the stones and remained of root of the plats.
6. Mix these samples to form one sample.
7. Use sieve with small pores (each experiments have its sieve pores diametprs).
8. Use clean and sterile tools ex; clean nylon sac.
9. Immediate study and examination of the samples as soon as possible and avoid storage. Otherwise store in cool place.

Isolation and Enumeration of Soil Microorganism

Soil is variable environment with divers' microbial community consist of bacteria, actinomyceytes, molds, yeast, algae and protozoa.

Necessary to use different types of culture media due to differences in dietary requirements for each type of microorganism to be isolate.

Note: culture media used the following according to the type of microorganisms to be isolate

1- Enumeration of bacteria used Nutrient agar

2- Enumeration of Actinomycetes used jensen media, characterized Actinomycetes isolated in dishes as dry and dusty or chalky Also characterized dishes distinctive odor similar to odor earth after rain.

3- Enumeration of fungi used sahouraud media.

There are two main methods of direct plate counting:

Spread plate method and pour plate method:

A. The spread plate method

Consists of evenly spreading the diluted sample over agar plate. Using this method yields colonies that form on the surface of the agar.

Procedure:

1. make serial dilution of microorganism sample in series of tubes containing D.W.
2. transfer 0.Iml from last dilution of microorganism culture-by pipette.
3. put it on the center of an agar plate.
4. moist spreader with alcohol and sterilize by flaming.
5. spread the sample on agar plat by spreader.
6. sterilize it again.
7. incubate the plate at 37°c for 24 hours, and then examine and count the present colonies distributed throughout the agar.

Note:

1- count plates which show only about 30-300 colonies.
2- Used colony counter to enumerate the colonies

Determine No. of bacterial cells in soil sample from equation:

$$No.ef\ hacterial\ cells\ /Iml = No.of\ colonies \times inverted\ dilution \times 10$$

B. The pour plate method

A volume of 1 ml of the diluted sample is put into a sterile Petri plate, and then melted agar is poured in and mixed with the sample. Using this method allows for a larger volume of the diluted sample. This method yields colonies colonies throughout the agar (growing both on the agar and in the agar, not just on the surface.

Procedure:

1. Put agar media in water bath in 45°c to liquefied.
2. Add 1 gm of sample to first tube and make serial dilution from one to another tube.
3. transfer 1ml from last dilution of microorganisms culture by pipette then put in sterile petri dish.

4. Pour melted agar and mixed with the dilution sample.

5. Leave petri dish to solidify.

6. Incubate the plate at 37°c for 24 hour.

Determine No. of bacterial cells in soil sample from equation:

$$No.of\ bacterial\ cells/1gm\ moist\ soil = No.of\ colonies\ inverted\ dilution$$

The unit of measurement here (CFU) Colony forming unit where the colony may be the yields of the growth and multiplication of a single cell or more.

Aerobiology

Aerobiology has been defined as the study of aerosolization, aerial ransmission, and deposition of biological materials.

Air spore in different layers of atmosphere, bioaerosol

A collection of airborne biological particles is called a bioaerosol. Bioaerosols are generated by a wide variety of natural and human-made processes including coughing, sneezing, wave action, splashes, cooling towers, ventilation systems, etc. Inhalation, ingestion, and dermal contact are routes of human exposure to airborne microorganisms, but inhalation is the predominant route that results in adverse human health effects. Airborne Legionella pneumophila, Mycobacterium tuberculosis, and some pathogenic viruses are known to be transmitted by (aerosols. Asthma, hypersensitivity pneumonitis and other respiratory illnesses are also associated with exposure to bioaerosols. Deterioration of building materials, offensive odors, and adverse human health effects are associated with microbial contamination of indoor environments, such as residences, offices, schools, health care facilities, enclosed agricultural structures (barns and crop storage areas) industrial facilities and recycling facilities.

Sources:

There are two basic sources of bioaerosol:

1- **Natural sources** are mainly soil and water, from which microorganisms are being lifted up by the movement of air, and from organisms such as fungi, that produce huge amounts of spores that are dispersed by the wind. Therefore, there are always a given number of microorganisms in the air, as a natural background. It is estimated, that the air is considered to be clean, if the concentration of bacteria and fungi cells does not exceed 1000/m^3 and 3000/m^3 respectively. This latter statement is only true when the concentration of microorganisms consists of saprophytic organisms, not pathogenic organisms. If

the concentration of microorganisms in the air exceeds the above values, or contains microorganisms dangerous to humans, then such air is considered to be microbiologically polluted.

2- **Living sources** of bioaerosols related to human activity, are more important than the natural sources. The emissions from these dangerous due to the following two reaaons: They may **distribute pathogenic microorganisms**, They often C**ause a high increase of microorganisms in the air**, significantly exceeding the natural background sources are The most important sources of bioaerosol emission are:
    - Agriculture and farming-food industry.
    - Sewage treatment plants.
    - Waste management.

Infectious airborne diseases

The mucous membrane of the respiratory system is a specific type of a gateway' for most airborne pathogenic microorganisms. Susceptibility to infections is increased by dust and gaseous air-pollution, e.g. SO_2 reacts with water that is present in the respiratory system, creating H_2SO_4. This irritates the layer of mucous. Consequently, in areas of heavy air pollution, especially during smog, there is an increased rate of respiratory diseases. Bioaerosols may, among other things, carry microbes that penetrate organs via the respiratory system. After settling, microbes from the air may find their way on to the skin or, carried by hands, get into the digestive system (from there, carried by blood, to other systems, e.g. the nervous system). Fungi that cause skin infections, intestinal bacteria that cause digestive system diseases or nervous system attacking enteroviruses are all examples of the above.

Viral diseases

After penetrating the respiratory system with inhaled air, particles of viruses reproduce inside the cuticle cells of both the upper and lower respiratory system. After reproduction some of the viruses stay inside the respiratory system causing various ailments (runny nose, colds, bronchitis, pneumonia), whereas others leave the respiratory system o other organs (e.g. chickenpox viruses attack the skin). The most noteworthy viruses are:

- Influenza (*orthomyxoviruses*)
- Influenza, measles, bronchitis, mumps and pneumonia among newoor (*paramyxoviruses*)
- German measles (similar to *paramyxoviruses*)
- Colds (rhinoviruses and *koronaviruses*)

- Cowpox and true pox (pox type viruses)
- Chickenpox (cold sore group of viruses).
- Foot-and-mouth disease (picorna type viruses)
- Meningitis, pleurodynia (enteroviruses)
- Sore throat, pneumonia (adenoviruses)

Bacterial diseases

Similarly to viruses, some bacteria that find their way to the respiratory system may also cause ailments of other systems. Especially staphylococcus infections assume various clinical forms (bone marrow, inflammation, skin necrosis, intestinal inflammation, pneumonia). Often, a susceptible base for development of various bacterial diseases is first prepared by viral diseases, e.g. staphylococcus pneumonia is usually preceded by a flu or mumps. Bacterial airborne diseases include:

- Tuberculosis (*Mycobacterium tuberculosis*).
- Pneumonia (*staphylococcus*, *pneumococci*, *Streptococcus pneumonia*.
- Scarlet fever, laryngitis (*streptococcus*).
- Inflammation of upper and lower respiratory system and meningitis (*Haemophilus influenzae*).
- Whooping cough (chromatobars of Bordetella pertussis).
- Diphtheria (*Corynebacterium diphtheriae*).
- Legionnaires disease (chromatobars of Legionella genus, among others *L.pneumophila*).
- Nocardiosis (oxygen actinomycetes of *Nocardia* genus).

Fungal diseases

Many potentially pathogenic airborne fungi or the so-called saprophytes live in soil. They usually have an ability to break down keratin (keratinolysis) - difficult to decompose proteins found in horny skin formations, e.g. human or animal hair, feathers, claws. Some of the keratinolytic fungi, the so-called dermatophytes, and cause mycosis of the outer skin (dermatosis), as the breakdown of keratin enable them to penetrate the epidermis. Other fungi, after penetrating the respiratory system, cause deep mycosis (organ), e.g. attacking lungs.

The following are examples of airborne fungi diseases:

- **Mycosis** (*Microsporum racemosum*)
- **Deep mycosis**: aspergillosis (*Aspergillus fumigatus*), **eryptococcus** (*Cryptococcus neoformans*).

Protozoan diseases

Some protozoa, which are able to produce cysts that are resistant to dehydration and solar radiation, may also infect humans by inhalation. The most common example of the above is: Pneumocystis carinii which causes pneumonia. Dangers connected with pathogenic bioaerosols do not concern only human diseases. Other significant diseases are those that attack cultivated plants or farm animals.

The following are example of the above:

Blight - grain disease caused by Puccinia graminis

Allergy

Allergy is a changed, hypersensitive reaction of the person or animal to some substances called allergens.

Causes
Actually, it's an immunologic reaction, in which a needless production o antibodies by B lymphocytes (mainly IgE and IgG immunoglobulinsl occurs as a hypersensitive response to penetration of antigens (called the **allergen**). Excessively produced immunoglobulins combine with allergens, **which cause:**

A release of various compounds (e.g. histamines) from mast cells. The released compounds induce inflamed reactions in the form of bronchus asthma or hay fever; Cause damaged tissue at the place of contact, - allergic pulmonary alveoli inflammation (e.g. the so-called farmer's lung, or mushroom breeder's lung).

Many microbes exist as allergens. Besides these, there are other allergenic factors such as anemophilous pollens (e.g. grass), small arachnids (mites) as well as biological dust particles of feathers, hair or droppings)

Microorganisms differ in their allergenic influences. The strongest allergens are mold fungi, thermophilus actinomycetes, as well as Gram negative chromatobars. The strength of allergenic bioaerosols depends not only on the type of microorganisms but also on 1) their A type of allergic reaction induced by biological aerosols 2) depends on the type of allergens that cause it as well as, 3) the size of its particles as it determines the degree of penetration into the respiratory system:

Particles larger than 10µm, held in the nasal cavity, cause hay fever (e.g. fungi spores, grass pollen) Particles of diameter between 4-10µm, held in bronchi, cause asthma (e.g. fungi spores of Cladosporium) Particles less than 4µm that penetrate alveoli, besides asthma induce allergic inflammation of pulmonary alveoli (fungi spores of Aspergillus and Penicillium).

Endotoxin in air and its quantification and hazards

Poisoning/intoxication are caused by toxins that are produced by some microorganisms. Endotoxins and mycotoxins are the most significant types of toxins in polluted air.

Endotoxins are the components of Gram-negative bacterial cell walls (A lipid fragment of lipopolysaccharides LPS outer membrane).

They demonstrate toxic (and allergenic) effects on mammals. After being inhaled into the lungs, they cause acute inflammation of the lungs. Mycotoxins are produced by various mold fungi. The most common ones are:

Aflatoxins produced by *Aspergillus flavus*. These compounds (there are several types of them) demonstrate strong toxic, mutagenic, carcinogenic, teratogenic (cause malformation in a fetus) actions. Most often they lead to food poisonings, however it has also been indicated, that inhaling dusts which contain aflatoxins may bring about tumours of the liver and the respiratory system.

Sampling techniques

Initially, the exposure of solid biological media to air provided the air microorganisms for study. Sterile nutrient media plates were exposed to the air by lifting the glass cover and exposing the media surface to air for a given period of time. Spores and a few vegetative cells, which settled out on the media surface, found the media suitable for rapid growth, allowing them to be examined in detail. While this technique is reasonable for a qualitative measure of the microbes in the immediate locality, it was not suitable for quantitative evaluation of microbes in the air. Later W. F. Wells at Harvard University developed an air sampler that allowed quantitative measurement of the microorganisms in air. The Wells air sampler was a combination centrifuge that sucked in air at a measured flow rate and forced the suspended particles onto the surface of biological media that coated the surface of clear glass centrifuge tubes. As the centrifugal action of the sampler pushed the microbial particles against the media surface, the microbes found an environment for rapid growth. Based on initial studies on the survival of bacteria in the air, Wells and Stone found that bacteria could survive for a sufficient period to allow pathogenic bacteria to be transmitted from person to person through the air. Then the scientists development of the membrane filter for collecting air samples for microbial growth. Membrane filters were cellulose acetate filters of specific pore sizes to allow retention of different size particles. Measured volumes of air were drawn through the membrane filters to capture all particles of a certain size. By using a series of different filters a spectrum of particle sizes could be measured. After passing the desired amount of air through the membrane filter, the membrane filter was placed on top of a porous pad containing specific microbiological media to stimulate the growth of the desired microbes.

Central heating and air conditioning

The development of central heating and air conditioning systems has created problems that are stimulating new interest in air microbiology. Houses and office buildings are being constructed tighter to prevent significant energy losses. Heated air in the winter and cool air in the summer are recirculated on a semicontinuous to continuous basis for maximum efficiency. Various types of filters are placed ahead of the circulation fans to remove particulates from the recycled air. Most of the simple filters trap large particles. A few specialized filters will remove small particles, such as bacteria. In large buildings electrostatic precipitators are used to remove microbial particles from the air. Electrostatic precipitators use high voltage, about 50,000 volts, across a series of flat metal plates to pull the tiny charged particles onto the plate surfaces. Figure 12-1 illustrates the removal of bacteria by an electrostatic precipitator. The velocity of airflow, the spacing between plates, the area of the plates, and the number of plates are important design parameters. Periodically, the metal plates are cleaned to remove the attached particles. Since bacteria and colloidal particles in the air tend to be negatively charged, all of the tiny particles are removed together onto the positively charged plates. Electrostatic precipitators can be very efficient in removing microorganisms from the air.

Nitrogen Fixation

Nitrogen-fixing bacteria is an example of mutualism, it mean bacteria capturing nitrogen.

All life forms need nitrogen. The "amino" part of amino acids comes from the words amine ($-NH_2$) and ammonia (NH_3), both of which, you'll notice, prominently feature nitrogen.

Do you know why people lost at sea can die of thirst, despite being surrounded by an ocean? That's because the ocean water isn't drinkable. It's WAY too salty. Plants are kind of in the same situation. Except instead of water, it's nitrogen.

Plants are surrounded by nitrogen. It makes up 80% of the atmosphere! However, they can't use it in that form. They have no way to access that nitrogen directly. Higher organisms depend upon bacteria to fix nitrogen. Bacteria are able to convert nitrogen in the air (N_2) into ammonia (NH_3) that plants can take up and use.

Of course, nitrogen-fixing bacteria wouldn't normally make any more ammonia than they need. Why should they? Plants have developed ways to coax the bacteria to share. Certain plants have developed special organs in their roots called root nodules. These organs accommodate gobs of nitrogen-fixing bacteria inside, providing safe, food-filled places in exchange for nitrogen.

While several pairs of plant and bacterial species have developed relationships like this, the best-studied relationships are between legume plants (including alfalfa, clover, and soy) and bacteria of the genus *Rhizobium*.

Not all plants form these relationships, though. Many rely on nitrogen from decaying matter. Remember crop rotation? It's the process of switching the crops we grow in a particular field from year to year. People have known for a long time that planting legumes every few years would lead to better growth of other crops in ensuing years. Now we know that's because the bacteria in legume roots are fertilizing the soil with nitrogen-containing compounds.

Nitrogen cycle

Nitrification Process:

Nitrification is the biological oxidation, is formally by a two-step process, in the first step oxidation ammonium to nitrite and in the second step the oxidation of the nitrite to nitrate. Nitrification is a second and important step in the nitrogen cycle in soil as converts of soil ammonia to nitrates, compounds usable by plants.

$$NH_4 \xrightarrow[\text{Notrosococcus}]{\text{Nitrosomona}} NO_2 \xrightarrow[\text{Nitrococcus}]{\text{Nitrobacters}} NO_3$$

Nitrification is an aerobic process performed by small groups of autotrophic bacteria different microbes are responsible for each steps. several bacteria of ammonia- oxidizing bacteria (AOB) , including *Nitrosomonus*, *Nitrosospora*, and *Nitrosococcus*.

In the second step, nitrite is oxidized to nitrate, by groups of nitrite - oxidizing bacteria (NOB), including *Nitrobacter*, *Nitrococcus Nitrospira* .

Nitrifying bacteria:

Are chemoautotrophic or chemolithotrophs (family Nitrobacteraceae) depending on the genera (*Nitrosomonas*, *Nitrosococcus*, *Nitrobacter*, *Nitrococcus*) bacteria that grow by consuming inorganic nitrogen compounds. Many species of nitrifying bacteria have complex internal membrane systems that are the location for key enzymes in nitrification: ammonia monooxygenase which oxidizes ammonia to hydroxylamine, and nitrite oxidoreductase, which oxidizes nitrite to nitrate.

Nitrosomonas and Nitrobacter are gram negative, mostly rod-shaped, microbes ranging between 0.6-0.4 microns in length. They are obligate aerobes and cannot multiply or convert ammonia or nitrites in the absence of oxygen.

Isolation and detection ammonia oxidizing bacteria:

Procedure:

1- Suspend 1 gm of soil sample in 9 ml of Allen I broth (contains (NH4), SO, as ammonia source).

2- Incubate tubes at 28C for a week.

3- Mix 1ml microbial suspension with an equal volume of reagent

A (salfanilic acid, acetic acid) and reagent

B (a-naphtholamine ,acetic acid).

4- Let for a few seconds, formation of red colored deposit illustrates releasing of No_2 as a result of nitrification process.

Isolation and detection Nitrite oxidizing bacteria:

Procedure:

1- Follow previous procedure, but substitute Allen II broth instate of Allen I broth is contains $NaNo_2$ as nitrite source for detection of released No_2

2- Mix 1ml microbial suspension with drops of nitrate reagent (Diphenylamin DPA).

3- Formation of blue colored deposit demonstrate releasing of No3.