Lab.1

Soil Microbiology

Soil is the brittle part of the hemisphere which formed due to different physicals and chemicals factors.

The soil differ in its texture regarding to its content of stones , grits and sand. The percentage of these content in the soil affect actively in the presence and viability of the M.O.

As we know the M.O need to quantity of water named (Active water) to continue its biological activities. So its spread and density will be high in the heavy soils comparing to its presence and spread in the light soils .Also the contents of the soil in organic & inorganic compound consider one of the main factors that affect the presence and the viability in the soil.

Factors which effect on the types and distributions of M.O. in the soil :

1- Type of the soil:

The types of the M.O, it's numbers and forms differ according to mechanical soil structure and the heavy soils are more rich in the M.O. than the sandy soils.

2- Light:

Most of M.O prefer to keep away from the light except the alge which prefer to life on the surface of the soil or near it.

3-Ventilation:

Most of the M.O of the soil are aerobic which mean its grow only in air present.

Some of them are an aerobic which mean that it’s growth stop when the air is presented. Another type is facultative (it can grow in the presence or absence of air).

4- Humidity:

Is essential to vitalize the soil but it differ to the degree of their tolerance to the dryness.

5- Temperature:

Most of the M.O. can live in the all type of the soils, but some of them like low or moderate temperature. The another type of M.O.like high temp. with soil rich in organic materials.

6- Acidity:

Most of the M.O.in the soil prefer neutral pH for their living, and Some prefer to live in acidity or alkaline Soil.

7- Types and Quantitative of the nutrient:

The M.O.of the soil are either parasitic, predator or opportunistic.

Types of the M.O. and its distributions in the soils :

1- Protista:

They are primitive M.O. Characterize by poor evolution. They play a role in the biochemical changes which break down the organic materials to its original metals.

2- Prokaryote:

Its nucleus M.O not Surround by nuclear membrane it is include the bacteria which is the smallest regarding to the size. and proliferation by simple division, the cells are either cocci, bacilli or spiral. live single or group, and forms colonies.

3- Viruses:

Its spread in the soil but it quickly loss its viability because of absences of the parasite (host) in the soil which is essential to its life.

4- Eukaryta:

Its nucleolus M.O has nuclear membrane and its either unicellular or multicellular , Its highly spread in the soils. Include fungi , alge and protozoa.

5- Microphona:

Its small M.O include primitive and some nematode and flat worms, Most of them are depend on another M.O for nutrition.

The role of M.O in the soil

Beneficial effects:

The small M.O break down the remnant of the animal and plant organic materials and help it's lyses and convert it to beneficial form for plant nutrition.

Harmful effects:

Some of the M.O in the soil cause many diseases to the human and animals and these M.O contaminate the soil via irrigation and dead infected animals, ex: bac. which cause tetanus and anthrax.

Collection of soil sample

1- Take in the consideration type of the soil fertilized or non fertilized soil, salty or not .

2- Remove the superficial layer of the soil because it's contaminated by so many environmental factors results from human activities , exposure to UV 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 simple must collected randomly.

5- Clean the samples from stones and remnant of the root of the plants.

6- Mix these to form one sample.

7- Use sieves with small pores (each experiments has it's sieve pores diameters).

8- Use clean and sterile tools , ex; clean nylon sac.

9- Immediate study and exanimation of the samples as soon as possible and avoid storage otherwise store in cool place.

Lab.2

Study of some physicals and chemicals criteria of the soil

1- **Physical Criteria :**

A-Temperature: The temp. of the soil effect on the type and numbers of M.O according to the different seasons.

B-Humidity: The amount of the water in the soil affect by many factors ex: Structure of the soils temp.

Procedure :

1- Weighing clean, empty and dry watch class or petri dish.

2- Weighing 10g of the soil and put it in the petri dish then put the petri dish in the oven at 105^{C} for 8-12 hr.

3- Take the sample directly to the desiccator containing $Cacl_2$ for 1hr or more to withdraw the ruminate of the water or the vapor.

4- Weighing the Sample and the watch glass directly after drying then do the following equation :

$$\text{Relative humidity} = \frac{\text{Weight of petri dish with hummed sample - Weight after desiccation}}{\text{Weight after desiccation}} \times 100$$

2- Chemical criteria:

A - Measuring the pH: It's measured the hydrogen ion concentration in the soil by two method:

1. Electric method:

by using pH meter, The pH meter has electrode that insert into the soil suspension, the scale range from (1-14) .

2.Colorimetric Method:

In this may we use chemicals sensitive indicators to the pH variation.

Procedure :

1- Take 1g of the soil and put in clean test tube with 5ml of Kcl as buffer. Close the test tube and shake sample by use vortex.

2- Leave the test tube to precipitate the soil then filter the supernatant .

3- Take 3 drops to 3 holes in porcelain Plate

*****The first hole as a control (Just soil suspension).

*****The second hole contains 1drope of soil suspension and 1drop of methyl red indicator.

*****The 3rd hole contain 1drop of the soil suspension. and 1drop of promothymal blue indicator.

4- Mix the soil suspensions with the indicators and estimate the pH according to the color which formed. according to the table:

Indicator	**Color**	**pH-value**
Methyl Red 0.02% (AI coholic)	Red	4.5
	Red orange	5-5.5
	Yellow	6.5
	Green Light	6.5-7
Bromothymal blue 0.04% (Aqueous)	Green. Blue	7-7.5
	Blue	8

Note: This method is outdated and not currently supported.

Lab 3

Isolation and Enumeration of Soil Microorganism

Soil is variable environment with divers‘ microbial community consists of bacteria, actinomycetes , 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 isolated:

1- Enumeration of bacteria used Nutrient agar .

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

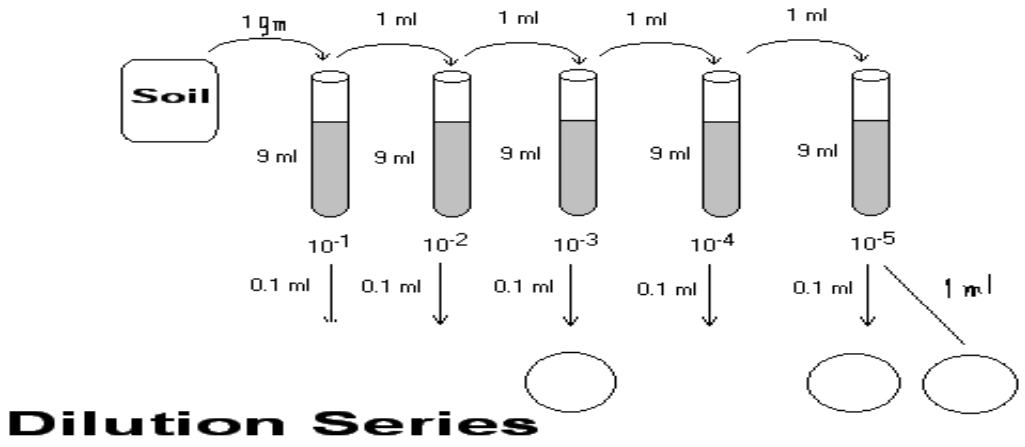
3 – Enumeration of fungi used sabouraud media.

There are two main methods of direct plate counting: spread plate method and pour plate method :

1-The spread plate method consists of evenly spreading the diluted sample over an 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 0.1ml from last dilution of microorganism culture by pipette.
3. put it on the centre 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.



Dilution Series

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. of bacterial cells /1ml= No. of colonies × inverted dilution ×10

2-The pour plate method, a volum of 1 ml of the diluted sample is put into a sterile petri plate, then melted agar is poured in and mixed with the sample. This method yields colonies that form colonies throughout the agar (growing both on the agar and in the agar , not just on the surface.

<u>procedure</u> :

1. Put agar media in water bath in 45°c. to liquefied .
2. Add 1gm of sample to first tube and make serial dilution from one to another tube.
3. transfer 1ml from last dilution of microorganism 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

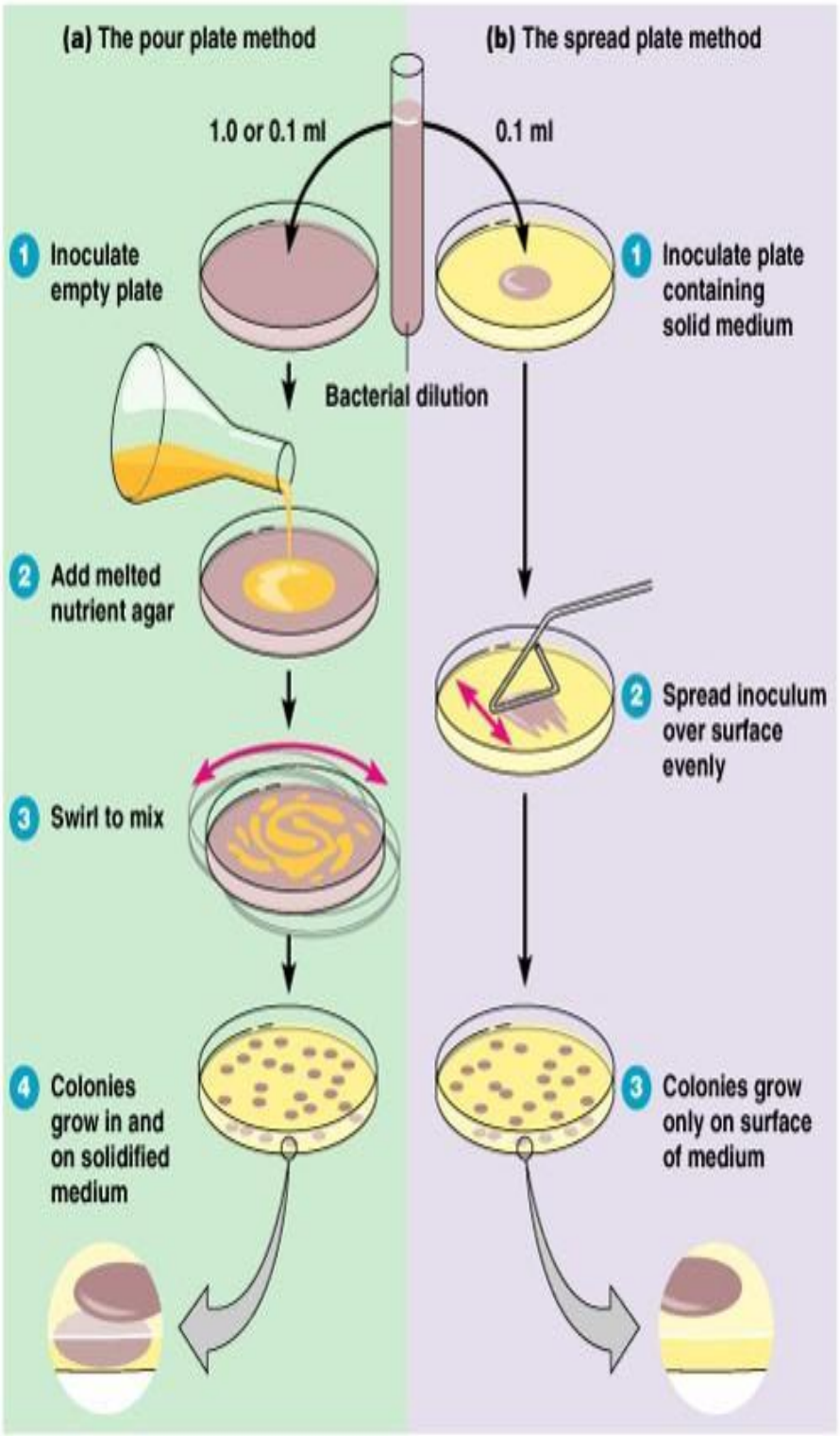
Determine No. of bacterial cells in soil sample from equation:

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

No. of bacterial cells /1gm dry soil = <u>No. of colonies ×inverted dilution</u>

Dry weight of 1gm soil sample

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.



Lab 4

General Examination of microorganisms

- Rossi and Cholodny Buried Slid Technique

The distribution of microorganisms in soil is heterogeneous. Microbes need nutrients and water to survive and these resources are not evenly distributed in soil. The structure of soil is composed of particles of inorganic and organic matter and the pores in between these particles. The pore spaces may be filled with water or air. Bacteria are mostly found attached to particles growing in small micro-colonies wherever nutrients can be found , Filamentous organisms such as actinomycetes and fungi found too.

One way to visualize how microbes are distributed in soil is to use the soil contact slide method developed by Rossi and Cholodny (1938). In this technique, glass slides are buried in soil and incubate for ~2 weeks. Bacteria and fungi attach to the glass as though it was a mineral particle and grow on the surface.

Procedure :

1-Weight 100 g of soil and placed in the clean container , if the soil is dry, moisten with water and adding (5 to 15 ml).

2- Make a slit in the soil and put the glass slide covered with media (semi solid) vertically in the Soil .

3 - Record the information on the glass slide .

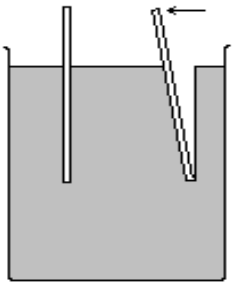
4 - Covered the cans and incubated at room temperature for week-2weeks.

5- Remove the slide after Incubation by tilting it backwards so that the top of the slide is not scraped off.

6- Pulls slide and washed with water carefully to remove soil particles and clean the bottom of the slide with a moist paper towel.

7- Drying slide by flame for fixation and then staining by Gram stain or Carbol Fuchsin or methyl blue and washed with water and leave to dry.

8- Examine the slide under a microscope and identify the microorganisms in the soil



Rossi and Cholodny Buried Slid Technique.

Lab :5

Isolation of Fruiting Gliding Bacteria

Soil is the main source for the isolation of most genera of bacteria. According to the method designed by scientist Stanier, one of the most efficient method for isolation strain that analyst cellulose from gliding bacteria , such as *Polyangium* , *Chondromyces* and *Nannocystis*.

One of the important described diagnostic for gliding bacteria is form fruiting bodies which invested successfully for their Isolation, This method Include use medium (Staniers mineral salt agar) provider with cellulose in the form of filter paper sterilized or powder as a only source of carbon's, showed as bright areas with yellow colors or orange or brown and even black on the filter paper around the patch dirt after incubation, accompanied with clear areas of decomposition. We can see thick fruiting bodies and mucus in mashed cellulose .

Procedure:

1- Preparation Culture medium (Staniers mineral salt agar) .

2- Put 5 grams of prepared soil sample in a sterile dish by sterile spatula then drops of cyclohexamide solution 50 mg / liter and mix well so that sufficient quantity added to formation a thick paste.

3- Divided the sample into small pieces and then distributed on the surface of the filter paper placed on the Culture medium .

4- Incubate the plate in incubator at a temperature 28 C for a (21-7) days. We must provide moist conditions , by put container filled with water in the incubator .

5- On the third day examined the plate by dissecting microscope at 40X- 20X to see fruiting bodies .

Lab : 6

Microbial decomposition of crude oil

There are many types of bacteria, fungi, and algae have the enzymatic ability to consumes petroleum hydrocarbons as the only source of carbon and energy and convert it into carbon dioxide and water, gas, as well as cellular materials, such as proteins and nucleic acids. This phenomenon have positive aspects where they can disposed of environmental pollution with oil and petroleum products.

These organisms play an important role in the treatment of oil pollution problem, dependent susceptibility microorganisms in the analysis of hydrocarbon compounds to the nature hydrocarbon compounds and proportions of oil and petroleum products, where crude oil contains hydrocarbon saturated, aromatic compounds and asphaltic an oxidized varying degrees by different microorganisms .

Such as bacteria:- ***Pseudomonas , Nocardia, Polyangium*.**

fungi :- ***Aspergillus*** .

yeast :- **Candida.**

Many factors affect the speed of analysis of the oil the most important : surface area exposed, the numbers of microorganisms , components and the type of oil , the time of exposure, aeration , temperature, nutrients , Ph and organism environment. the biodegradation of oil requires a suitable mixture of microorganisms , contact with oxygen and large amounts of nitrogen and phosphorus compounds and small amounts of other essential elements for the growth of all microorganisms.

To complete the analysis process efficiently required a mixture of different microbes, because oil is composed of a variety of hydrocarbons, therefore every microorganism specialist to analyze a specific type of hydrocarbon.

Procedure:

1- Distribute media a(mineral salts broth) at a rate of 50 ml in flasks (200 ml capacity) .

2- Add the oil 4% sterile with filtration (filter diameter of 0.45 M) and then inoculated flasks with 1g soil and leave some flasks without inoculated (control). The flasks closed to prevent volatilization of oil.

3- Incubated flasks in the incubator vibrating at 150 r/ min and a temperature of 28 C for 5 weeks ,then observe the growth , The oil drops in the broth have some changes; as reduced quantity, and discolored to dark, and looked great size because of microbial cells aggregate around it, microorganisms become in contact directly with the oil drops, which facilitates the transition of the drops and pass through the cell membrane to the cytoplasm.

As well as microorganisms growth led to mixing oil with salt components of the media ,then emulsified for short duration ,because the secretion of emulsified substances work to reduce the viscosity of the oil and then mixed with the aqueous phase.

Lab 7

Melanin pigment production

Many of microorganisms in the soil have the ability to produce the enzyme (Tyrosinase),which used to oxidize the amino acid tyrosine (that located in the environmental of the microorganism as a single source of carbon and energy) to Melanin according to the following equation:

$$\text{L – Tyrosine} \xrightarrow{\text{Tyrosinase (oxidation)}} \text{Melanin (dark pigment)}$$

Types of microorganisms producing enzyme:

bacteria: Streptomyces , Polyangium .

Fungi : Epicoccum .

Procedure :

1-Preparation the media (Tyrosine agar) add (0.5%) of the L- Tyrosine to the nutrient agar.

2-Make serial dilution soil to (10^{-4}).

3- Transfer (0.1) ml of soil diluted in agar plate , then spread the sample on agar plate by spreader.

4-Incubated at a temperature (28 c) for (2- 3)day .

5- After incubation observed the change the color of the media to brown or brown - greenish or black as an indication of the production of melanin.

Melanin is produced by the oxidation of the amino acid tyrosine by Tyrosinase .

Melanins also protect microorganisms, such as bacteria and fungi, against stresses that involve cell damage such as UV radiation from the sun, protects against damage from high temperatures and oxidizing agents, melanins appear to play important roles in virulence and pathogenicity by protecting the microbe against immune responses of its host.

Semi carotenoids pigments production

Many microorganisms that live on surfaces exposed to lightuse semi carotenoids, a pigment found in the membrane cytoplasm have the ability to absorb light energy and provide protection from photo oxidation, which kills microorganisms.

Some carotenoids are produced by bacteria to protect from oxidative immune attack. The golden pigment that gives some strains of Staphylococcus aureus their name (aureusis = golden) is a carotenoid called staphyloxanthin.

Bacteria :Micrococcus roseus

Procedure :

1-Transfer (1 g) soil to the 50 ml of M-Medium broth in bottle.

2-Incubate at temperature (28 c) for (4-6 days) in the light.

3-After Incubation observed change the color of the media to yellow, then orange with time as an indication of the production of pigments carotenoids compared with the control which grown in the dark.

Lab 8

The role of soil microorganisms in changes elements

Carbon cycle

Elements such as carbon, nitrogen and oxygen at different rates are Compose contents cells of living organisms (animal, plant, microorganisms) and these elements exist in the environment in the form of free molecules or atoms in organic compounds and organic.

Microorganisms possess many enzymatic systems (natural or stimulating) decomposition of complex compounds to simple exploited by other organisms and products are needed to feed the growth of these organisms .

Degradation of Cellulose :

Degrades cellulose by microorganisms may take one of two way: either fully analyzed and the resulting sugar glucose, which is used source of carbon and energy in this case, has a microbiology system enzymatically complete as what is shown in the following equation:

$$\text{Cellulose} \xrightarrow{\text{Cellulose}} \text{Cellobiose} \xrightarrow[\text{B-glucosidase}]{\text{Cellobiase}} \text{glucose}$$

Or some microorganisms do not have the full system as result of cellobiose.

Procedure :

1. Weigh (1g) of soil sample under study and added to the tube contain the media specialized to isolating bacteria that decomposing cellulose (Doubose cellulose media) with a filter papers as the source of carbon (and cellulose). Put in the middle (paraffin wax is placed to isolate anaerobic bacteria decomposing cellulose).
2. Incubated tubes at 28 C for one to four weeks.

3. Examine the filter papers in the test every week until the appearance of disintegration as a shot holes as well as the appearance of yellow spots on the filter papers which indicates the decomposition of cellulose.
4. Make suspended by cut piece of filter paper and put in clean petri dish with some of D.W and then work tinge on a clean glass slide, and leave it to dry and then pigmentation with dye Gram stain then washed with water and leave to dry and examined under a microscope.

Bacterial cellulose decomposing :

Bacillus sp. ⟶ Spore forming Gve+ , *Clostridium sp.* Gve+

Pseudomonas sp. ⟶ Gve- عصيات مفردة

Cytophage sp. ⟶ Gve- مغزلية الشكل

Molds & Fungi of cellulose decomposing:

Aspergillus sp. Penicillium sp. Fusarium sp. Trichoderma sp.

Filamentous bacteria from cellulose decomposing:

Micromonospora sp. Nocardia sp. Streptomyces sp.

<u>Degradation of pectin :</u>

Pectin found in the Middle lamella in the cells of plants and some algae, associated with calcium carbonate, which gives plant cells mainstay constant. Some microorganisms analysis of pectin such as bacteria *Erwinia carotovora* and mold species belonging to the genus *Fusarium* sp.

Microorganisms excreted enzyme analyst pectin(Pectinase) which analyzes the pectin and thus lose the plant cell-strong hardness.

<u>Procedure :</u>

1. works soil suspension by adding (1 g) of soil to test tube container to (9) ml of sterile distilled water.

2. Cut potato tubers and pollinate their surfaces amount of soil stuck, placed in sterile glass dishes.
3. Add the amount of sterile water to the dishes during the incubation to avoid drying happen.
4. Note Maceration phenomenon (the following week) to the potatoes because of the decomposition of pectin in the middle lamella between plant cells because most of the installation of the walls is composed of pectin.

Degradation of starch :

Starch degrades by microorganisms to monosaccharaides and disaccharides (glucose), which has analyzed the starch enzymes such as amylase (α- , β -amylase and Isoamylase) these enzymes affect the bonds of sugar units consisting of starch .

Procedure :

1. Works soil suspension by adding (1) g of soil to test tube container to (9) ml of sterile distilled water.
2. Use media (Starch agar) and pour in sterile glass dishes (use this medium to isolate the bacteria analyzed for starch).
3. Add (0.1) ml of selected diluted to the medium surface after it is spreading on the middle surface using a sterile glass spreader .
4. Incubated at a temperature of 28 C for a week.
5. Observed growth on the surface of the dish and to identify the bacteria analyzed for starch add to one of the dishes drops of iodine solution after two minutes note the presence of a halo transparent clear zone around the colonies evidence of the production of enzymes, either areas distant from the colonies made up the blue color as a result of iodine interaction with starch .
6. Work swabs of growth (Gram stain) and examined under a microscope to identify the microbiology analyst for starch.

   Bacteria : *Bacillus sp. Clostridium sp. Micrococcus sp.*

   Molds & Fungi : *Aspergillus sp. Rhizopus sp. Fusarium sp*.

Lab : 9

Nitrogen Cycle

Nitrogen fixation :

This process in which nitrogen (N_2) in the atmosphere is convert into ammonia (NH_3) fixed form ,by number of soil microorganisms are capable to production an enzyme called nitrogenase which needs to metal molybdenum and iron to complete the process. This process is essential for all forms of life because nitrogen is required to biosynthesize basic building blocks of plants, animals and other life forms, e.g., nucleotides for DNA and RNA and amino acids for proteins.

Two types of bacteria are responsible for fixing nitrogen :

Family 1 : Azotobacteriaceae ex. *Azotobacter*.

Family 2 : Rhizobiaceae ex. *Rhizobium.*

Some types of bacteria can fixing nitrogen but in less efficient such as *Clostridium* , *Klebsiella* ,Cyanobacteria .

Nitrogen fixation can possible to get in two ways:

1- Symbiotic Nitrogen fixation . ex. *Rhizobium.*
2- Non-Symbiotic Nitrogen fixation (free living). ex. . *Azotobacter* sp. , Azospirillum

Symbiotic Nitrogen fixation :--- ex. *Rhizobium*

Nitrogen fixation through nodule formation in Leguminous plants

Leguminous plants bearing nodules on its root as a result of injury root hairs with bacteria *Rhizobium* , In large numbers inside this root nodules . Bacteria be parasite on plant where they get the necessary energy for the growth and fixed nitrogen that useful for plant . The symbiotic Bacteria Rhizobia (from the Greek words Riza = Root and Bios = Life)

Procedure:

1- Take plant called sweet clover (*Melilotus*) from soil.
2- Cut roots & wash it well with tap water to remove soil particles .
3- Cut large pink or red nodules true nodules (neer the root) , False nodules Located far from root.
4- Put true nodules on clean slide and smash it with another slide , leave it to dry and stain with gram pigment .

5- Examine under oil immersion , *Rhizobium* appear look like latin letters Y , X, Z, T .

Non-Symbiotic Nitrogen fixation (free living):

The fixation of free nitrogen in the soil can gets by most microorganisms living freely or outside the plant cell that called non-symbiotic N_2 fixation. It is performed by the aerobic and anaerobic bacteria and blue green algae.

The process involved microorganism in soil free-living *Azotobacter chroococcum* bacteria , this bacteria G-ve, motile , often pleomorphic ranging from rode to cocci shapes , strict aerobic, the colonies appear like sticky mucus because they around by cyst , when bacteria grown on agar media form large convex , mucoid colonies , with brownish color, growth in PH (7.2-8) .To isolation this bacteria use (Nitrogen –free glucose medium) ,this contains sources carbon, molybdenum .

This bacteria produce two type of pigments :Use to distinguish between 6 types of *Azotobacter*

1-Water- soluble pigments. (It's the important because it spread in culture medium).

2-Water-in soluble pigments.

Procedure:

1-Transfer 1gm of soil to Nitrogen –free glucose broth medium that packaged in glass bottles.

2- Close the bottles not tight to provide enough aeration and get N_2 from air .

3- Incubate at 30c for 7days in horizontal position to increase the surface area of culture medium that exposed to the air .

4- after incubation :transfer 0.1 ml from Nitrogen –free glucose broth to Nitrogen –free glucose agar and streaking to show colony shape and color water- soluble pigment.

Lab : 10

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 convertes of soil ammonia to nitrates, compounds usable by plants.

$$NH_4 \xrightarrow[\text{Nitrosococcus}]{\text{Nitrosomonas}} NO_2 \xrightarrow[\text{Nitrococcus}]{\text{Nitrobacter}} NO_3$$

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 *Nitrosomonas*, *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 $(NH_4)_2SO_4$ as ammonia source) .

2- Incubate tubes at $28C^0$ 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 illustrate 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_3 .

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

3- formation of blue colored deposit demonstrate releasing of No_3.

Lab :11

Isolate microorganisms producing antibiotics from soil :

Soil is one of the important sources of microorganisms used in the laboratory of life to impose production of various types of enzymes, antibiotics and other growth factors such as vitamins, amino acids and others.

Featuring microbiology in natural environments almost the existence of many of the interrelationships among them, these relationships may be useful or harmful.

Examples of useful relations for these organisms are commensalism where there are two types of organisms exploit one from the other for the purpose of obtaining the necessary growth substances has one the parties utilized and the other does not hurt, either harmful relations, it is the competition between the two to obtain the necessary growth substances may stop one types second growth produces toxic materials and these materials are antibiotics where antagonism is defined as :

the production of toxic substances that have the ability to stop the growth of microorganisms by other microorganisms, leading to the inability of these organisms on living in the nearby region of them.

Where many of the microorganisms in the soil produces retardant materials for the growth of many other microorganisms.

Of microbiology producing antibiotics are bacteria and fungi and Actinomycetes. Where is Actinomycetes of the most important soil organisms that create antibiotics .

Which produces streptomycin types belonging to the genus streptomyces, which inhibits a wide range of positive and negative bacteria Gram stain .

And also cycloheximide, which inhibits the eukaryote organisms, which is used as a disincentive to the growth of fungi at isolating the bacteria count .

Among the most important genus Actinomycetes producing antibiotics :

Streptomyces , Actinomycetes , Nocaradia , Micromonospora

Among the most important genus of bacteria producing antibiotics :

Bacillus , *Pseudomonas* , that have the ability to produce pyocianin and others.

The type of most important genus fungi producing antibiotics :

Aspergillus , Fusarium , Pencillium , Trichoderma

Crowded plate technique :

This method is used for the purpose of isolating the bacteria that have the ability to produce antibiotics, as well as to isolate the bacteria producing some growth factors and this method are as follows:

1- works soil suspension by adding (1) g of soil to test tube container to (9) ml of sterile distilled water.
2- Spread selected dilutions on agar surface (solid) then incubate at 37C for 24hr .
3- After incubation period we select the plate that growth present on it .
4- Select 5 well isolated colonies from plate and then suspend in 1 ml D.W in 5 tubes.
5- Cultivate isolated pathogenic bacteria on nutrient agar and then make pores on it by saqib corky.
6- Put 0.1 ml from suspend in each pores.
7- Incubate the plate at 37 C for 24 hr .
8- After incubation we observe clear zone around the pores as indicate to producing antibiotics.