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(Deemed to be University)
(Under Section 3 of UGC Act 1956)

DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
(Established Under Section 3 of UGC Act, 1956)
Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

I M. Sc MICROBIOLOGY - 2019 – 2021 Batch

ENVIRONMENTAL AND AGRICULTURAL MICROBIOLOGY (19MBP204)

SEMESTER – II **4H – 4C**

SYLLABUS

Instruction Hours / week: L: 4 T:0 P: 0 **Marks:** Internal: **40** External: **60** Total:**100**

End Semester Exam:
3Hours

COURSE OBJECTIVES

- To educate the students about concepts of designs of water distribution systems, sewer networks, working principles and design of various physical, chemical and biological treatment systems of water and wastewater.
- To study about the biofertilizers, plant disease and increasing soil fertility.

COURSE OUTCOME (CO'S)

1. This course will provide the student insights into these invaluable areas of Environmental microbiology, which play a crucial role in determining its future use and applications inenvironmental management.
2. Students able to know detailed idea about biofertilizer production and plant disease.

UNIT I- Aquatic environment

Microbiology of water - water pollution and water borne pathogens. Bacteriological examination of water, indicator organism. Microbiology of sewage. Chemical and biochemical characteristic of sewage. Methods of sewage treatment - physical screening, chemical, biological (sludge digestion; activated sludge, aerating filters, oxidation pond).

UNIT II - Microbiology of air and Bioremediation

Microbial contaminants of air, sources of contamination, microbial indicators of air pollution. Enumeration of bacteria in air. Air samplers and sampling techniques. Air sanitation. Bioremediation of air pollutants. Bioleaching – recovery of metal from ores – oxidation of minerals – testing for biodegradability.

UNIT III – Microbes in agriculture

Importance of microbes in agriculture, Current agriculture problems. Bacterial diseases of agricultural crops -pathogens, symptoms and control measures with reference to paddy, cotton, maize, tomato, citrus, mango and potato. Plant protection –phenolics – phytoalexins and related compounds. Bioinsecticides – bacterial and fungal brief note.

UNIT – IV - Biological nitrogen fixation

Symbiotic and non-symbiotic microorganisms, root nodule formation, nitrogen fixers, hydrogenase, Nitrogenase, *Nif* gene regulation. Biochemistry of nitrogen fixation, Rhizosphere- R: S ratio, Interaction of microbes with plants. Bioconversion of agricultural wastes.

UNIT V- Biofertilizers and Biocontrol

Application of biofertilizers and biomanures – A combination of biofertilizer and manure applications with reference to soil, seed and leaf sprays. Plant growth promoting microorganisms- Myzorrhizae,
Rhizobia, Azosprillum, Azotobacter, Azolla, Frunkia, Blue green algae, Phosphate- solubilizers fluorescent *Pseudomonas*. Laboratory and field application; Cost-benefit analysis of biofertilizer and biomanure production. Biocontrol and its application: Biofungicides, bionematicides and Biopesticides.

SUGGESTED READINGS

1. Saxena., and Sanjai., (2015). *Applied Microbiology*. Springer,Germany.
2. Denise., G.A., Sarah, S., and Deborah, A., (2015). *Nester's Microbiology*. McGraw-Hill Education
3. SubbaRao, N.S. (1999). *Biofertilizers in Agriculture and Agroforestry*. Oxford and IBH, NewDelhi.
4. Rangaswami, G., and Bhagyaraj, D.J., (2001). *Agricultural Microbiology*. (2nded.). Prentice Hall, New Delhi.
5. Rao, N.S. (1995). *Soil Microorganisms and plant Growth*. Oxford and IBH Publishing Co., New Delhi.
6. Pelzar, M.J., and Reid, M., (2003). *Microbiology*. (5thed.). Tata McGraw-Hill, NewYork.
7. Reinheimer, G. (1991). *Aquatic Microbiology*. (4thed.). John Wiley and Sons, NewYork.
8. Deniel, J.C. (1996). *Environmental aspects of microbiology*, British Sun Publication,Chennai.
9. Abbasi, S.A. (1998). *Environmental pollution and its control*. Cogent International publishers, Pondicherry.
10. Sen, K., and Ashbolt, N.J., (2010). *Environmental Microbiology: Current Technology and Water Applications.*
11. Josdand, S.N. (1995). *Environmental Biotechnology*. Himalaya Publishing House,Bombay.
12. Maier, R.M., Pepper, I,L., and Gerba, C.P., (2009). *Environmental Microbiology*. (2nded.). Elsevier Publisher.
13. Metcalf, R.L., and Luckmann, W.H., (1994). *Introduction to insect pest management.* (3rded). John Willey and Sons,Inc.
14. Atlas, R.M., and Bartha, M., (2000). *Microbial Ecology - Fundamental and Applications*. (3rded.). Redwood City CA. Benjamin/Cumming Science Publishing Co., NewDelhi.
15. Maier, R.M., Pepper, I.L., and Gerba, C.P., (2000). *Environmental Microbiology*. (1sted.). Academic Press, NewYork.
16. Mitchell, R. (1992). *Introduction to Environmental Microbiology*; Prentice Hall. Inc. Englewood Clifs- NewJersey.
17. Motsara, M.R., Bhattacharyya, P., and Srivastava, B., (1995). *Biofertilizer-*

Technology, *Marketing and Usage*. *Fertilizer Development and Consultant Organization*, NewDelhi.

18. Madigan, M.T., Martinka, M., Parker, J. and Brock, T.D. 2000. Twelth Edition, Biology Microorganisms, Prentice Hall, New Jerry. 5.

Mark Wheelis, 2010. Principles of Modern Microbiology, Jones & Bartlett India Pvt. Ltd., New Delhi

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I M.Sc Microbiology **COURSE NAME: Environmental and Agricultural Microbiology**

COURSE CODE: 19MBP204 **BATCH-2019-2021**

S.No.	Lecture Duration (Period)	Topics to be Covered	Support Materials
		Unit - I	
1.	1	Microbiology of water - water pollution and water borne pathogens	T1 : 15.2-15.6
2.	1	Bacteriological examination of water	T2:142-144
3.	1	Indicator organism	T4 : 105-106
4.	1	Microbiology of sewage	T4 : 108-110
5.	1	Chemical and biochemical characteristic of sewage	T2 :144-145
6.	1	Methods of sewage treatment - physical screening, Chemical screening	T2 :234-236
7.	1	Biological screening-sludge digestion; activated sludge	T2 :236-237
8.	1	Aerating filters, oxidation pond.	T2 :240-241
9.	1	Revision	
		Total No. of Hours Planned for Unit-I	**9**
		Unit - II	
1.	1	Microbial contaminants of air, Sources of contamination	T3:919-926
2.	1	Microbial indicators of air pollution	A1: 251-258
3.	1	Enumeration of bacteria in air, Air samplers and sampling techniques	T2 : 118-121 , W2
4.	1	Air sanitation	A2 : 93-118
5.	1	Bioremediation of air pollutants	A3

6.	1	Bioleaching – recovery of metal from ores	T2: 228-229
7.	1	Oxidation of minerals	T2:32- 35
8.	1	Testing for biodegradability.	R2: 533-535
9.	1	Recapsulation	
Total No. of Hours Planned for Unit-II			**9**
Unit - III			
1.	1	Importance of microbes in agriculture	W4
2.	1	Current agriculture problems	W5
3.	1	Bacterial diseases of agricultural crops- Paddy	W6
4.	1	Bacterial diseases of agricultural crops-Cotton, Maize	W7
5.	1	Bacterial diseases of agricultural crops- Tomato, Citrus	T5 :309-311, R1: 23-29
6.	1	Bacterial diseases of agricultural crops- Mango and Potato	T5 :307-310
7.	1	Plant protection –phenolics compounds and phytoalexin and related compounds	T6:137
8.	1	Bioinsecticide- bacterial insecticide.Fungiside	T3: 92-98
9.	1	Recapitulation and Discussion of important questions	
Total No. of Hours Planned for Unit-III			**9**
Unit - IV			

1.	1	Symbiotic and non-symbiotic microorganisms	T4:398-412
2.	1	Root nodule formation, nitrogen fixers	T4: 399-403
3.	1	Hydrogenase and Nitrogenase enzymes	T4: 407-414
4.	1	*Nif* gene regulation	T4:415-417
5.	1	Biochemistry of nitrogen fixation	T4: 403-405
6.	1	Rhizosphere- R:S ratio	W3
7.	1	Interaction of microbes with plants	T4: 415-416
8.	1	Bioconversion of agricultural wastes	T4: 416-418
9.	1	Recapitulation and Discussion of important questions	
		Total No. of Hours Planned for Unit-IV	**9**
		Unit - V	
1.	1	Application of biofertilizers and biomanures	T5:396
2.	1	A combination of biofertilizer and manure applications with reference to soil, Seed and leaf spray	T9: 217-219
3.	1	Plant growth promoting microorganisms- Myzorrhizae and Rhizobia	T7:166-228
4.	1	Azosprillum, Azotobacter and Azolla,	T7: 132-133,160,164
5.	1	Frunkia and Blue green algae	T4:178-180, R2: 157-163
6.	1	Phosphate- solubilizers fluorescent *Pseudomonas*	T5: 244-246

7.	1	Laboratory and field application; Cost-benefit analysis of biofertilizer and biomanure production	T9:298-300
8.	2	Biocontrol and its application: Biofungicides, bionematicides and Biopesticides.	T8: 117-119 T7: 376-381
9.	1	Recapitulation and Discussion of important questions	
10.	1	Recapitulation and Discussion of previous semester question papers	
Total No. of Hours Planned for Unit-V			**11**
Total No. of Hours Planned			**52**

Textbook:

1. SubbaRao, N.S. (1999). *Biofertilizers in Agriculture and Agroforestry*. Oxford and IBH, NewDelhi.
2. Rangaswami, G., and Bhagyaraj, D.J., (2001). *Agricultural Microbiology*. (2nd ed.). Prentice Hall, New Delhi.
3. Rao, N.S. (1995). *Soil Microorganisms and plant Growth*. Oxford and IBH Publishing Co., New Delhi.
4. Pelzar, M.J., and Reid, M., (2003). *Microbiology*. (5th ed.). Tata McGraw-Hill, NewYork.
5. Reinheimer, G. (1991). *Aquatic Microbiology*. (4th ed.). John Wiley and Sons, NewYork.
6. Deniel, J.C. (1996). *Environmental aspects of microbiology*, British Sun Publication,Chennai.

Unit 1

Aquatic environment - microbiology of water - water pollution and water borne pathogens. Bacteriological examination of water, indicator organism. Microbiology of sewage. Chemical and biochemical characteristic of sewage. methods of sewage treatment - physical screening, chemical, biological (sludge digestion; activated sludge, aerating filters, oxidation pond).

An aquatic ecosystem is an ecosystem in a body of water. Communities of organisms that are dependent on each other and on their environment live in aquatic ecosystems. The two main types of aquatic ecosystems are marine ecosystems and freshwater ecosystems

Marine ecosystem

Marine ecosystems cover approximately 71% of the Earth's surface and contain approximately 97% of the planet's water. Marine ecosystems can be divided into many zones depending upon water depth and shoreline features.

The oceanic zone is the vast open part of the ocean where animals such as whales, sharks, and tuna live.

The benthic zone consists of substrates below water where many invertebrates live.

The intertidal zone is the area between high and low tides; in this figure it is termed the littoral zone.

Freshwater ecosystem

Freshwater ecosystems cover 0.80% of the Earth's surface and inhabit 0.009% of its total water. They generate nearly 3% of its net primary production. Freshwater ecosystems contain 41% of the world's known fish species.

There are three basic types of freshwater ecosystems

Lentic: slow moving water, including pools, ponds, and lakes.

Lotic: faster moving water, for example streams and rivers.

Wetlands: areas where the soil is saturated or inundated for at least part of the time

This biota have a size range (maximum linear dimension) up to 200 mm, and vary from viruses through bacteria and archea, to micro-algae, fungi and protozoa.

Functions

Aquatic ecosystems perform many important environmental functions. For example, they recycle nutrients, purify water, attenuate floods, recharge ground water and provide habitats for wildlife. Aquatic ecosystems are also used for human recreation, and are very important to the tourism industry, especially in coastal regions.

The health of an aquatic ecosystem is degraded when the ecosystem's ability to absorb a stress has been exceeded. A stress on an aquatic ecosystem can be a result of physical, chemical or biological alterations of the environment. Physical alterations include changes in water temperature, water flow and light availability. Chemical alterations include changes in the loading rates of biostimulatory nutrients, oxygen consuming materials, and toxins. Biological alterations include over-harvesting of commercial species and the introduction of exotic species. Human populations can impose excessive stresses on aquatic ecosystems. There are many examples of excessive stresses with negative consequences. Consider three. The environmental history of the Great Lakes of North America illustrates this problem, particularly how multiple stresses, such as water pollution, over-harvesting and invasive species can combine. The Norfolk Broadlands in England illustrate similar decline with pollution and invasive species. Lake Pontchartrain along the Gulf of Mexico illustrates the negative effects of different stresses including levee construction, logging of swamps, invasive species and salt water intrusion.

Microbiology of water

Water microbiology is concerned with the microorganisms that live in the water, or those that can be transported from one habitat to another by water. The improvement of pathogen detection methodology is an important issue for the efficient prevention of waterborne outbreaks. Bacterial populations are a natural component of lakes, rivers, streams and other aquatic systems. Over 60 genera of bacteria are present in aquatic systems and numbers can range from forty thousand to over twelve million bacterial cells in an amount of water. The immense numbers of these small organisms can have an enormous impact on processes that occur in aquatic ecosystems such as carbon, nitrogen, and sulphur transformations. They can also have an impact on the quality of water by controlling the amount of oxygen in the water and causing diseases in aquatic organisms as well as in humans. Naturally some microorganisms have learned to live on or in the human body. Many of these microorganisms do not harm, and are even beneficial because they compete with other microorganisms that might cause diseases. A few microorganisms can cause disease in humans. These microorganisms are called pathogens.Some pathogens live out their lives in the soil and water and only cause disease under unusual circumstances. The microorganism that causes tetanus (a bacterium named Clostridium tetani).This microorganism lives normally in the soil. Other pathogens are more closely associated with humans and other warmblooded animals. These pathogens are transmitted from one organism to another by direct contact, or by contamination of food or water However, the presence of other disease causing microbes in water is unhealthy and even life threatening. For example, bacteria that live in the intestinal tracts of humans and other warm blooded animals, such as *Escherichia coli, Salmonella*, *Shigella*, and *Vibrio*, can contaminate water if feces enter the water. Contamination of drinking water with a type of *Escherichia coli* known as O157:H7 can be fatal. The contamination of the municipal water supply of Walkerton, Ontario, Canada in the summer of 2000 by strain O157:H7 sickened 2,000 people and killed seven people.

The intestinal tract of warm-blooded animals also contains viruses that can contaminate water and cause disease. Examples include rotavirus, enteroviruses, and coxsackievirus.

Another group of microbes of concern in water microbiology are protozoa. The two protozoa of the most concern are *Giardia* and *Cryptosporidium*. They live normally in the intestinal tract of animals such as beaver and deer. *Giardia* and *Cryptosporidium* form dormant

and hardy forms called cysts during their life cycles. The cyst forms are resistant to chlorine, which is the most popular form of drinking water disinfection, and can pass through the filters used in many water treatment plants. If ingested in drinking water they can cause debilitating and prolonged diarrhea in humans, and can be life threatening to those people with impaired immune systems. *Cryptosporidium* contamination of the drinking water of Milwaukee, Wisconsin with in 1993 sickened more than 400,000 people and killed 47 people.

Many microorganisms are found naturally in fresh and saltwater. These include bacteria, cyanobacteria, protozoa, algae, and tiny animals such as rotifers. These can be important in the food chain that forms the basis of life in the water. For example, the microbes called cyanobacteria can convert the energy of the sun into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water is also an important food source for other forms of life.

A variety of microorganisms live in fresh water. The region of a water body near the shoreline (the littoral zone) is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use light as energy thrive in this zone. Further away from the shore is the limnitic zone. Photosynthetic microbes also live here. As the water deepens, temperatures become colder and the oxygen concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green sulfur bacteria, which can grow without oxygen, dominate. Finally, at the bottom of fresh waters (the benthic zone), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

Saltwater presents a different environment to microorganisms. The higher salt concentration, higher pH, and lower nutrients, relative to freshwater, are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., *Pseudomonas* and *Vibrio*). Also, in 2001, researchers demonstrated that the ancient form of microbial life known as archaebacteria is one of the dominant forms of life in the ocean. The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

Another microorganism found in saltwater are a type of algae known as dinoflagellelates. The rapid growth and multiplication of dinoflagellates can turn the water red. This "red tide" depletes the water of nutrients and oxygen, which can cause many fish to die. As well, humans can become ill by eating contaminated fish.

Water can also be an ideal means of transporting microorganisms from one place to another. For example, the water that is carried in the hulls of ships to stabilize the vessels during their ocean voyages is now known to be a means of transporting microorganisms around the globe. One of these organisms, a bacterium called *Vibrio cholerae*, causes life threatening diarrhea in humans.

Drinking water is usually treated to minimize the risk of microbial contamination. The importance of drinking water treatment has been known for centuries. For example, in pre-Christian times the storage of drinking water in jugs made of metal was practiced. Now, the anti-bacterial effect of some metals is known. Similarly, the boiling of drinking water, as a means of protection of water has long been known.

Chemicals such as chlorine or chlorine derivatives has been a popular means of killing bacteria such as *Escherichia coli* in water since the early decades of the twentieth century. Other bacteria-killing treatments that are increasingly becoming popular include the use of a gas called ozone and the disabling of the microbe's genetic material by the use of ultraviolet light. Microbes can also be physically excluded from the water by passing the water through a filter. Modern filters have holes in them that are so tiny that even particles as miniscule as viruses can be trapped.

An important aspect of water microbiology, particularly for drinking water, is the testing of the water to ensure that it is safe to drink. Water quality testing can be done in several ways. One popular test measures the turbidity of the water. Turbidity gives an indication of the amount of suspended material in the water. Typically, if material such as soil is present in the water then microorganisms will also be present. The presence of particles even as small as bacteria and viruses can decrease the clarity of the water. Turbidity is a quick way of indicating if water quality is deteriorating, and so if action should be taken to correct the water problem.

In many countries, water microbiology is also the subject of legislation. Regulations specify how often water sources are sampled, how the sampling is done, how the analysis will be performed, what microbes are detected, and the acceptable limits for the target microorganisms in the water sample. Testing for microbes that cause disease (i.e., *Salmonella typhymurium* and *Vibrio cholerae*) can be expensive and, if the bacteria are present in low numbers, they may escape detection. Instead, other more numerous bacteria provide an indication of fecal pollution of the water. *Escherichia coli* have been used as an indicator of fecal pollution for decades. The bacterium is present in the intestinal tract in huge numbers, and is more numerous than the disease-causing bacteria and viruses. The chances of detecting *Escherichia coli* are better than detecting the actual disease causing microorganisms. *Escherichia coli* also had the advantage of not being capable of growing and reproducing in the water (except in the warm and food-laden waters of tropical countries). Thus, the presence of the bacterium in water is indicative of recent fecal pollution. Finally, *Escherichia coli* can be detected easily and inexpensively.

Water pollution

Definition: Water pollution is characterized by certain observable disturbance in normal properties and functions of fresh water. Eg : includes offensive odour, bad taste etc.,

Water pollution occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds.

Surface water and groundwater have often been studied and managed as separate resources, although they are interrelated. Surface water seeps through the soil and becomes groundwater.

Ground water pollution

Ground water is considered to be safe and useful for drinking, agricultural and industrial purpose. The specific contaminants leading to pollution in water include chemicals and substances such fluoride, arsenic, nitrate etc., the concentration is the key in determining contaminant. High concentrations of naturally occurring substances can have negative impacts on aquatic flora and fauna and the substances are toxic to humans.

Surface water pollution

Surface water includes rivers, lakes and reservoirs, surface water is susceptible for pollution eg : industrial, domestic, agricultural etc.,

Nature of pollutants

Pollutants may be Dissolved, Suspended,Colloidal in state, they are further categorised as

Organic pollutants

Synthetic organic pollutants

Inorganic pollutants

Organic water pollutants include

Detergents

Disinfection by-products found in chemically disinfected drinking water, such as chloroform Food processing waste, which can include oxygen-demanding substances, fats and grease, Insecticides and herbicides, a huge range of organohalides and other chemical compounds, Petroleum hydrocarbons, including fuels (gasoline, diesel fuel, jet fuels, and fuel oil) and lubricants (motor oil), and fuel combustion byproducts, from storm water runoff, Tree and bush debris from logging operations.

Volatile organic compounds (VOCs)

Chlorinated solvents, which are dense non-aqueous phase liquids (DNAPLs), may fall to the bottom of reservoirs, since they don't mix well with water and are denser.Polychlorinated biphenyl (PCBs).Trichloroethylene. Perchlorate

Various chemical compounds found in personal hygiene and cosmetic products

Drug pollution involving pharmaceutical drugs and their metabolites.

Inorganic water pollutants include

Acidity caused by industrial discharges (especially sulfur dioxide from power plants)

Ammonia from food processing waste

Chemical waste as industrial by-products

Fertilizers containing nutrients--nitrates and phosphates—which are found in stormwater runoff from agriculture, as well as commercial and residential use[16]

Heavy metals from motor vehicles (via urban stormwater runoff)[16][17] and acid mine drainage

Silt (sediment) in runoff from construction sites, logging, slash and burn practices or land clearing sites.

Microbiological pollutant

Microbiological pollution is caused by wide range of microorganisms like bacteria, viruses , protozo, helminths can cause serious diseases that can lead to death.

Radioactive pollutant

Radioactive contamination, also called radiological contamination, is the deposition of, or presence of radioactive substances on surfaces or within solids, liquids or gases, where their presence is unintended or undesirable. E.g uranium, radium, thorium.

Water borne pathogens

Waterborne diseases are caused by pathogenic microorganisms that most commonly are transmitted in contaminated fresh water. Although the vast majority of bacteria are either harmless or beneficial, a few pathogenic bacteria can cause disease. Coli form bacteria, which are not an actual cause of disease, are commonly used as a bacterial indicator of water pollution. Other microorganisms sometimes found in surface waters that have caused human health problems include:

Burkholderia pseudomallei

Cryptosporidium parvum

Giardia lamblia

Salmonella

Norovirus and other viruses

Parasitic worms including the Schistosoma type

High levels of pathogens may result from on-site sanitation systems (septic tanks, pit latrines) or inadequately treated sewage discharges. This can be caused by a sewage plant designed with less than secondary treatment (more typical in less-developed countries). In developed countries, older cities with aging infrastructure may have leaky sewage collection

systems (pipes, pumps, valves), which can cause sanitary sewer overflows. Some cities also have combined sewers, which may discharge untreated sewage during rain storms.

BACTERIAL EXAMINATION OF WATER

The bacteriological examination of water is performed routinely by water utilities to ensure a safe supply of water for drinking, industrial and other domestic uses. The examination is intended to identify water sources which have been contaminated with potential disease causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery. In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms. The coliform group is comprised of Gram-negative, nonspore-forming, aerobic to facultatively anaerobic rods, which ferment lactose to acid and gas. Two organisms in this group include E. coli and Enterobacter aerogenes; however, the only true fecal coliform is E. coli, which is found only in fecal material from warm-blooded animals. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed until tests no longer detect E. coli. The three principal tests used for bacterial examinations are

Presumptive test

Confirmative test

Complete test

Standard water analysis

The Presumptive test

In the presumptive test, a series of lactose broth tubes are inoculated with measured amounts of the water sample. Gas production in any one of the tubes is presumptive evidence of the presence of coliforms. The most probable number (MPN) of coliforms in 100 ml of the water sample can be estimated by the number of positive tubes.

The confirmed test

If any of the tubes inoculated with the water sample produce gas, the water is presumed to be unsafe. In order the confirm the presence of coliforms, it is necessary to inoculate EMB (eosin methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram-positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. E. coli and E. aerogenes can be distinguished from one another by the size and color of the colonies. E. coli colonies are small and have a green metallic sheen, whereas E. aerogenes forms large pinkish colonies. If only E. coli or if both

E. coli and E. aerogenes appear on the EMB plate, the test is considered positive. If only E. aerogenes appears on the EMB plate, the test is considered negative. The reasons for these interpretations are that, as previously stated, E. coli is an indicator of fecal contamination, since it is not normally found in water or soil, whereas E. aerogenes is widely distributed in nature outside of the intestinal tract.

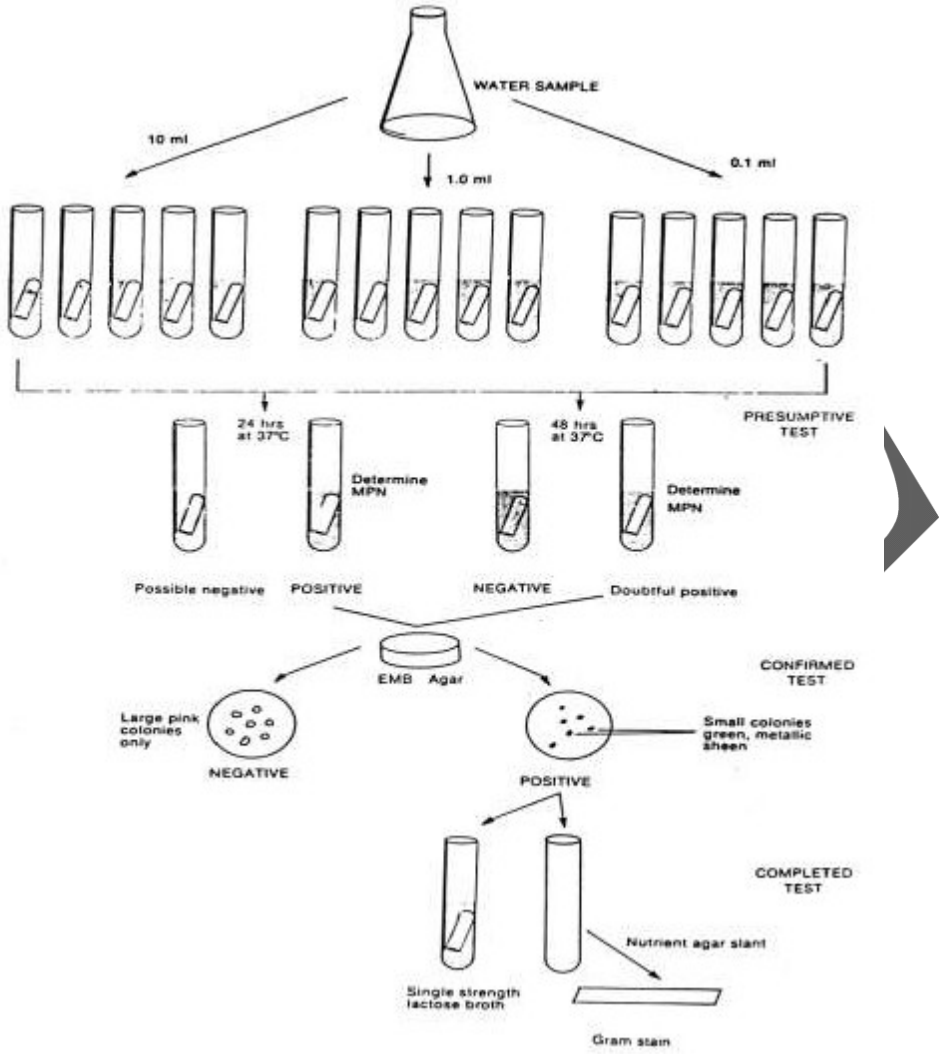
The completed test

The completed test is made using the organisms which grow on the confirmed test media. These organisms are used to inoculate a nutrient agar slant and a tube of lactose broth. After 24 hours at 37°C, the lactose broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant. If the organism is a Gram-negative, nonspore-forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample.

The water sample is inoculated in three tubes of lactose broth with 10 ml, three tubes with 1.0 ml and three tubes with 0.1 ml. Incubate all tubes at 37oC for 24 hours. Observe the number of tubes at each dilution that show gas production in 24 hrs. Reincubate for an additional 24 hours at 37°C. Inoculate an EMB plate with material from a tube containing gas. Invert and incubate the plate at 37°C for 24 hours. Observe EMB agar plates. A positive confirmed test is indicated by small colonies with dark centres and a green metallic sheen (E. coli). Inoculate a lactose broth tube and a nutrient agar slant with organisms from the EMB plate. Incubate the broth tube and agar slant at 37°C for 24 hours

MPN DETERMINATION FROM MULTIPLE TUBE TEST

NUMBER OF TUBES GIVING POSITIVE REACTION OUT OF			MPN Index per 100 ml.	95 PERCENT CONFIDENCE LIMITS	
3 of 10 ml. each	3 of 1 ml. each	3 of 0.1 ml. each		Lower	Upper
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1,300
3	3	1	460	71	2,400
3	3	2	1,100	150	4,800



Indicator organism

Cabelli (1977) noted that the best indicator organism should be the one whose densities correlate best with health hazards associated with one or several given types of pollution sources. The requirements for an indicator as follows:

The indicator should be consistently and exclusively associated with the source of the pathogens.

It must be present in sufficient numbers to provide an accurate density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable.

It should approach the resistance to disinfectants and environmental stress, including toxic materials deposited therein, of the most resistant pathogen potentially present at significant levels in the sources.

It should be quantifiable in recreational waters by reasonably facile and in expensive methods and with considerable accuracy, precision, and specificity.

Indicator organisms indicate that fecal pollution and presences of microbial pathogens. Total and fecal coliforms, and the enterocci - fecal streptocci are important indicator organisms. Coliform bacteria include all aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation. There are three groupings of coliform bacteria used as standards: total coliforms (TC), fecal coliforms (FC) and Escherichia coli. Total coliforms are the broadest grouping including Escherichia, Enterobacter, Klebsiella, and Citrobacter. These are found naturally in the soil, as well as in feces. Fecal coliforms are the next widest grouping, which includes many species of bacteria commonly found in the human intestinal tract. Usually between 60% and 90% of total coliforms are fecal coliforms. E. coli are a particular species of bacteria that may or may not be pathogenic but are ubiquitous in the human intestinal tract. Generally more than 90% of the fecal coliform are Escherichia coli.

Microbiology ofsewage

Wastewater, by its nature, is teaming with microbes. Many of these microbes are necessary for the degradation and stabilization of organic matter and thus are beneficial. On the other hand, wastewater may also contain pathogenic or potentially pathogenic microorganisms, which pose a threat to health. Microbes play an extremely important role in sewage treatment. It is largely through biological digestion that sewage is converted from a highly contaminated, infectious liquid into a relatively stable, inert sludge and a harmless effluent needing only chlorination before it may be discharged into a receiving stream, leaching bed, or other disposal area. There are two biological processes involved in sewage treatment.

Aerobic digestion is exemplified by the activated sludge process, in which the wastes from primary settling tanks are thoroughly aerated until active masses of microorganisms settle out as sludge, leaving a clear effluent of relatively low organic content. A portion of the sludge is returned and mixed with the incoming raw sewage, while the remainder is pumped to digester tanks. Anaerobic digestion is a slower process, which is typified by large digestion tanks, septic tanks, and cesspools. The main focus of wastewater treatment plants is to reduce the BOD (biochemical oxygen demand) and COD (chemical oxygen demand) in the effluent discharged to natural waters, meeting state and federal discharge criteria. Wastewater treatment plants are designed to function as "microbiology farms", where bacteria and other microorganisms are fed oxygen and organic wasteewage

Characteristics of Sewage:

Characterization of sewage is essential for an effective and economical waste management programme. It helps in the choice of treatment methods deciding the extent of treatment, assessing the beneficial uses of wastes and utilizing the waste purification capacity of

natural bodies of water in a planned and controlled manner. The wastes are generally characterized as follows:

(i) Physical characteristics: The physical characteristics are colour, odour, turbidity and temperature.

Colour: Fresh sewage is yellowish green or light brown. It is detected by naked eye.

Odour: Fresh sewage is odorless, In 3 to 4 hours, it becomes stale due to exhaust of oxygen in the sewage, mainly due to decomposition of sewage. It is measured by Threshold odour number (TON).

Turbidity: Sewage is generally turbid. If becomes more turbid due to floating matters like floating paper, grease, match sticks, fluid skins etc. Turbidity increases as sewage becomes stronger. Turbidity is measured by turbidity meter.

Temperature: The observation of temperature of sewage is useful in indicating solubility of oxygen, which affects transfer capacity of aeration equipment in aerobic systems and rate of biological activity. Normal temperature of sewage is higher than that of water. Raw temperature of sewage under Indian conditions is between 15-35°C, mostly 20°C.

(ii) Chemical characteristics: Chemical characteristics of sewage help us to find the stage of sewage decomposition, its strength and type of treatment required for safe disposal.

pH: The hydrogen ion concentration expressed as pH, it is valuable parameter in the operation of biological units. Generally pH of raw sewage is in range of 5.5 to 8.0.

Solid matter: Sewage water contains 0.05 to 0.1% of total solids. They are present in four forms

(a) Suspend solids
(b) Dissolved solids
(c) colloidal solids
(d) settle able solids
(e) Organic matter
(f) Inorganic matter.

Chloride content:

Chloride present in sewage from kitchens, urinals, bathrooms & industries. The normal limit is 120 mg/l. The maximum permissible limit is 250mg/l.

Nitrogen content: The presence of nitrogen indicates presence of organic matters. This exists in the form of nitrates, free NH_3 and aluminoides. Nitrate indicates intermediate stage of decomposition. Hence it helps to find the amount of treatment to be done. Natural oxidation prevents nitrates and nitrites.

Dissolved oxygen: Amount of oxygen gas dissolved in sewage water is called as dissolved oxygen. It has to be noted while discharging sewage into stream water. Dissolved content should be more than 4 ppm, else the fishes die and thus affects aquatic cycle. Fresh sewage contains DO in certain amount that gets depleted due to aerobic decomposition. When temperature increases, DO reduces. It is determined by winkler's method

. Biochemical oxygen demand and eutrophication

Organic material in wastewater originates from microorganisms, plants, animals, and synthetic organic compounds. Organic materials enter wastewater in human wastes, paper products, detergents, cosmetics, and foods. They are typically a combination of carbon, hydrogen, oxygen and nitrogen and may contain other elements.

The oxidation of organic materials in the environment can have profound effects on the maintenance of aquatic life and the aesthetic quality of waters. Biochemical oxidation reactions involve the conversion of organic material using oxygen and nutrients into carbon dioxide, water and new cells. The equation that expresses this is:

Organic material + O_2 + nutrients CO_2 + H_2O + new cells + nutrients + energy

It can be seen from this equation that organisms use oxygen to breakdown carbon-based materials for assimilation into new cell mass and energy. A common measure of this oxygen use is biochemical oxygen demand (BOD). BOD is the amount of oxygen used in the metabolism of biodegradable organics. If water with a large amount of BOD is discharged into the environment, it can deplete the natural oxygen resources. Heterotrophic bacteria utilize deposited organics and oxygen at rates that exceed the oxygen-transfer rates across the water surface. This can cause anaerobic conditions, which leads to noxious odors. It can also be detrimental to aquatic life by reducing dissolved oxygen concentrations to levels that cause fish to suffocate. The end result is an overall degradation of water quality.

Wastewater often contains large amounts of the nutrients, particularly nitrogen and phosphorous, which are essential for growth of all organisms and are typically limiting in the environment. Nitrogen is a complex element existing in both organic and inorganic forms. The forms of most interest from a water quality perspective are organic nitrogen, ammonia, nitrite, and nitrate. Phosphorous is found in synthetic detergents and is used for corrosion control in water supplies.

The introduction of large concentrations of these nutrients from untreated or improperly treated wastewater can lead to eutrophication. Eutrophication is the process by which bodies of water become rich in mineral and organic nutrients causing plant life, especially algae, to proliferate, then die and decompose thereby reducing the dissolved oxygen content and often killing off other organisms.

Chemical oxygen demand (COD): The amount of oxygen required to oxidize the organic matter present in sewage (both biologically active and inactive)

Generally COD is greater than BOD. Thus BOD/COD is always less than 1.

.(iii) Biological characteristics: The bacterial characteristics of sewage are due to the presence of micro-organisms, which include bacteria and other living micro-organisms such as algae, fungi, protozoa etc.

Pathogens: it Creates harm to humans, animals, crops.

Non-pathogens: it does not produce harm.

Aerobic bacteria: it survives in the presence of oxygen.

Anaerobic bacteria: Flourish in absence of oxygen.

Facultative bacteria: Survive with or without oxygen.

Most of the microorganisms help in decomposition of sewage.

Sewage Treatment:

Sewage, before being disposed of either in river or streams or on land, has to be treated for making it safe. The degree of treatment required depends on the characteristics of sewage & source disposal. Sewage can be treated in different ways.

The treatment process are often classified as

(i) Preliminary treatment

(ii) Primary treatment

(iii) Secondary treatment

(iv)Tertiary/final treatment (sometimes).

(i) **Preliminary treatment:** It consists of separating the floating materials like dead animals, tree branches, paper, and pieces of wood etc. and also heavy settable inorganic solids. It helps in removing oils and greases etc from sewage. This reduces BOD of waste water by about 15 to 30%. The processes used are

(a) Screening: To remove floating papers, rags, clothes etc.

(b) Grit chamber/Detritus sand: To remove grit and sand

(c) Skimming tanks: To remove oils and greases

(ii) **Primary Treatment**: It consists of removing large suspended organic solids. This is usually done by sedimentation in settling basins. The liquid effluent from primary treatment often contains a large amount of suspended organic material and has high BOD (about 60% of original). The organic solids, which are separated out in sedimentation tanks are stabilized by anaerobic decomposition in digestion tank or incinerated. The residue is used for landfills or soil conditioners.

Secondary Treatment:

Secondary treatment is biological process of very fine suspended matter, colloids and dissolved solids in sewage that comes from primary sedimentation tank.

The treatment stabilizes and makes the sewage completely harmless.

The unit process of secondary treatment are biological oxidation and synthesis through sewage filter or activated sludge process, converting sewage into heavier and bulkier and then allowing it to settle in secondary sedimentation tank.

The separated sewage sludge is decomposed anaerobically in sludge tank and the digested sludge is disposed off separately in sludge drying beds.

Difference between primary treatment and secondary treatment of sewage

(i) Primary treatment:

It is evident that the sewage as it arrives at the treatment plant would initially undergo primary treatment for the removal of heavy suspended matter such as solids, kitchen refuse, cloth, wastepaper etc.

Inorganic matter like sand, grit and other floating matter.

The primary treatment involves subjecting sewage subsequently to unit processes such as screening. Screening is the removal of grit and other floating matter and the remaining solids are removed through sedimentation process in primary sedimentation tank or clarifier. The primary treatment removes the physical impurities present in sewage along with solid matter. Sometimes preliminary treatment is also termed along with the primary treatment.

(ii) Secondary Treatment:

Secondary treatment involves further treatment of the effluent coming from the primary sedimentation tank. This is generally accomplished through biological decomposition of organic matter, under aerobic or anaerobic conditions. Bacteria get decomposed to fine organic matter, to produce clearer effluent.

(i) Aerobic biological unit: Under aerobic conditions eg: filters, aeration tanks, oxidation ponds, aerated lagoons

(ii) Anaerobic biological unit: Under anaerobic conditions eg: anaerobic lagoons, septic tanks etc.

Benefits of sewage treatment:

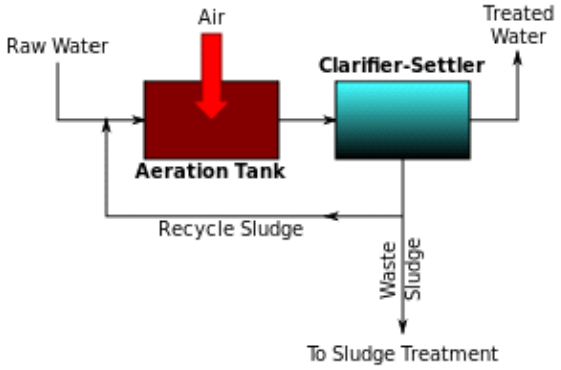
Save money by recycling a portion of waste water for use around garden.

Limit the impact of house waste on environment by becoming more self sufficient.

Protects precious source of ground water and saves rainwater in dams by recycling.

Reduce impact on municipal sewage system by installing domestic treatment system, particularly grey water treatment system.

Methods of sewage treatment



Activated sludge

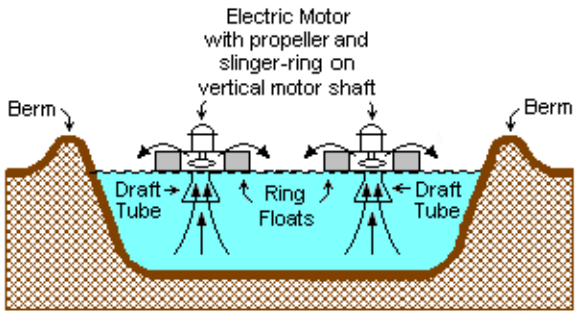
Activated sludge plants encompass a variety of mechanisms and processes that use dissolved oxygen to promote the growth of biological floc that substantially removes organic material. Biological floc, as mentioned above, is an ecosystem of living biota that subsists on nutrients from the inflowing primary settling tank (or clarifier) effluent. These mostly carbonaceous dissolved solids undergo aeration to be broken down and biologically oxidized or converted to carbon dioxide. Likewise, nitrogenous dissolved solids (amino acids, ammonia, etc.) are also oxidized by the floc to nitrites, nitrates, and, in some processes, to nitrogen gas through denitrification. While denitrification is encouraged in some treatment processes, in many suspended aeration plants denitrification will impair the settling of the floc and lead to poor quality effluent. In either case, the settled floc is both recycled to the inflowing primary effluent to regrow, or is partially 'wasted' (or diverted) to solids dewatering, or digesting, and then dewatering. This many times takes the form of the floating brown foam, Nocardia. While this so called 'sewage fungus' (it isn't really a fungus) is the best known, there are many different fungi and protists that can overpopulate the floc and cause process upsets. Additionally, certain incoming chemical species, such as a heavy pesticide, a heavy metal (eg.: plating company effluent) load, or extreme pH, can kill the biota of an activated sludge reactor ecosystem. Such problems are tested for, and if caught in time, can be neutralized.

Aerobic granular sludge

Activated sludge systems can be transformed into aerobic granular sludge systems (aerobic granulation) which enhance the benefits of activated sludge, like increased biomass retention due to high sludge settlability.

Surface-aerated basins

Many small municipal sewage systems in the United States (1 million gal./day or less) use aerated lagoons.Most biological oxidation processes for treating industrial wastewaters have in common the use of oxygen (or air) and microbial action. Surface-aerated basins achieve 80 to 90 percent removal of BOD with retention times of 1 to 10 days. The basins may range in depth from 1.5 to 5.0 metres and use motor-driven aerators floating on the surface of the wastewater. In an aerated basin system, the aerators provide two functions: they transfer air into the basins required by the biological oxidation reactions, and they provide the mixing required for dispersing the air and for contacting the reactants (that is, oxygen, wastewater and microbes). Typically, the floating surface aerators are rated to deliver the amount of air equivalent to 1.8 to 2.7 kg O2/kW·h. However, they do not provide as good mixing as is normally achieved in activated sludge systems and therefore aerated basins do not achieve the same performance level as activated sludge units.Biological oxidation processes are sensitive to temperature and, between 0 °C and 40 °C, the rate of biological reactions increase with temperature. Most surface aerated vessels operate at between 4 °C and 32 °C.



A TYPICAL SURFACE – AERATED BASIN

Note: The ring floats are tethered to posts on the berms.

Filter beds

Trickling filter beds are used where the settled sewage liquor is spread onto the surface of a bed made up of coke (carbonized coal), limestone chips or specially fabricated plastic media. Such media must have large surface areas to support the biofilms that form. The liquor is typically distributed through perforated spray arms. The distributed liquor trickles through the

bed and is collected in drains at the base. These drains also provide a source of air which percolates up through the bed, keeping it aerobic. Biological films of bacteria, protozoa and fungi form on the media's surfaces and eat or otherwise reduce the organic content. This biofilm is often grazed by insect larvae, snails, and worms which help maintain an optimal thickness. Overloading of beds increases the thickness of the film leading to clogging of the filter media and ponding on the surface. Recent advances in media and process micro-biology design overcome many issues with trickling filter designs.

Biological aerated filters

Biological Aerated (or Anoxic) Filter (BAF) or Biofilters combine filtration with biological carbon reduction, nitrification or denitrification. BAF usually includes a reactor filled with a filter media. The media is either in suspension or supported by a gravel layer at the foot of the filter. The dual purpose of this media is to support highly active biomass that is attached to it and to filter suspended solids. Carbon reduction and ammonia conversion occurs in aerobic mode and sometime achieved in a single reactor while nitrate conversion occurs in anoxic mode. BAF is operated either in upflow or downflow configuration depending on design specified by manufacturer.

Rotating biological contactors

Rotating biological contactors (RBCs) are mechanical secondary treatment systems, which are robust and capable of withstanding surges in organic load. RBCs were first installed in Germany in 1960 and have since been developed and refined into a reliable operating unit. The rotating disks support the growth of bacteria and micro-organisms present in the sewage, which break down and stabilize organic pollutants. To be successful, micro-organisms need both oxygen to live and food to grow. Oxygen is obtained from the atmosphere as the disks rotate. As the micro-organisms grow, they build up on the media until they are sloughed off due to shear forces provided by the rotating discs in the sewage. Effluent from the RBC is then passed through final clarifiers where the micro-organisms in suspension settle as a sludge. The sludge is withdrawn from the clarifier for further treatment.

A functionally similar biological filtering system has become popular as part of home aquarium filtration and purification. The aquarium water is drawn up out of the tank and then cascaded over a freely spinning corrugated fiber-mesh wheel before passing through a media filter and back into the aquarium. The spinning mesh wheel develops a biofilm coating of microorganisms that feed on the suspended wastes in the aquarium water and are also exposed to the atmosphere as the wheel rotates. This is especially good at removing waste urea and ammonia urinated into the aquarium water by the fish and other animals.

Membrane bioreactors

Membrane bioreactors (MBR) combine activated sludge treatment with a membrane liquid-solid separation process. The membrane component uses low pressure microfiltration or ultrafiltration membranes and eliminates the need for clarification and tertiary filtration. The membranes are typically immersed in the aeration tank; however, some applications utilize a separate membrane tank. One of the key benefits of an MBR system is that it effectively overcomes the limitations associated with poor settling of sludge in conventional activated sludge (CAS) processes. The technology permits bioreactor operation with considerably higher mixed liquor suspended solids (MLSS) concentration than CAS systems, which are limited by sludge settling. The process is typically operated at MLSS in the range of 8,000–12,000 mg/L, while CAS are operated in the range of 2,000–3,000 mg/L. The elevated biomass concentration in the MBR process allows for very effective removal of both soluble and particulate biodegradable materials at higher loading rates. Thus increased sludge retention times, usually exceeding 15 days, ensure complete nitrification even in extremely cold weather.

The cost of building and operating an MBR is often higher than conventional methods of sewage treatment. Membrane filters can be blinded with grease or abraded by suspended grit and lack a clarifier's flexibility to pass peak flows. The technology has become increasingly popular for reliably pretreated waste streams and has gained wider acceptance where infiltration and inflow have been controlled, however, and the life-cycle costs have been steadily decreasing. The small footprint of MBR systems, and the high quality effluent produced, make them particularly useful for water reuse applications.

Secondary sedimentation

The final step in the secondary treatment stage is to settle out the biological floc or filter material through a secondary clarifier and to produce sewage water containing low levels of organic material and suspended matter.

Tertiary treatment

The purpose of tertiary treatment is to provide a final treatment stage to further improve the effluent quality before it is discharged to the receiving environment (sea, river, lake, wet

lands,ground, etc.). More than one tertiary treatment process may be used at any treatment plant. If disinfection is practiced, it is always the final process. It is also called "effluent polishing."

Filtration

Sand filtration removes much of the residual suspended matter. Filtration over activated carbon, also called carbon adsorption, removes residual toxins.

Lagooning

Lagooning provides settlement and further biological improvement through storage in large man-made ponds or lagoons. These lagoons are highly aerobic and colonization by native macrophytes, especially reeds, is often encouraged. Small filter feeding invertebrates such as Daphnia and species of Rotifera greatly assist in treatment by removing fine particulates.

Nutrient removal

Wastewater may contain high levels of the nutrients nitrogen and phosphorus. Excessive release to the environment can lead to a buildup of nutrients, called eutrophication, which can in turn encourage the overgrowth of weeds, algae, and cyanobacteria (blue-green algae). This may cause an algal bloom, a rapid growth in the population of algae. The algae numbers are unsustainable and eventually most of them die. The decomposition of the algae by bacteria uses up so much of the oxygen in the water that most or all of the animals die, which creates more organic matter for the bacteria to decompose. In addition to causing deoxygenation, some algal species produce toxins that contaminate drinking water supplies. Different treatment processes are required to remove nitrogen and phosphorus.

Nitrogen removal

The removal of nitrogen is effected through the biological oxidation of nitrogen from ammonia to nitrate (nitrification), followed by denitrification, the reduction of nitrate to nitrogen gas. Nitrogen gas is released to the atmosphere and thus removed from the water.

Nitrification itself is a two-step aerobic process, each step facilitated by a different type of bacteria. The oxidation of ammonia (NH_3) to nitrite (NO_2^-) is most often facilitated by Nitrosomonas spp. ("nitroso" referring to the formation of a nitroso functional group). Nitrite oxidation to nitrate (NO_3^-), though traditionally believed to be facilitated by Nitrobacter spp. (nitro referring the formation of a nitro functional group), is now known to be facilitated in the environment almost exclusively by Nitrospira spp.

Denitrification requires anoxic conditions to encourage the appropriate biological communities to form. It is facilitated by a wide diversity of bacteria. Sand filters, lagooning and reed beds can all be used to reduce nitrogen, but the activated sludge process (if designed well) can do the job the most easily. Since denitrification is the reduction of nitrate to dinitrogen gas, an electron donor is needed. This can be, depending on the wastewater, organic matter (from faeces), sulfide, or an added donor like methanol. The sludge in the anoxic tanks (denitrification tanks) must be

mixed well (mixture of recirculated mixed liquor, return activated sludge [RAS], and raw influent) e.g. by using submersible mixers in order to achieve the desired denitrification.

Phosphorus removal

Phosphorus can be removed biologically in a process called enhanced biological phosphorus removal. In this process, specific bacteria, called polyphosphate-accumulating organisms (PAOs), are selectively enriched and accumulate large quantities of phosphorus within their cells (up to 20 percent of their mass). When the biomass enriched in these bacteria is separated from the treated water, these biosolids have a high fertilizer value. Phosphorus removal can also be achieved by chemical precipitation, usually with salts of iron (e.g. ferric chloride), aluminum (e.g. alum), or lime. This may lead to excessive sludge production as hydroxides precipitates and the added chemicals can be expensive. Chemical phosphorus removal requires significantly smaller equipment footprint than biological removal, is easier to operate and is often more reliable than biological phosphorus removal.

Disinfection

The purpose of disinfection in the treatment of waste water is to substantially reduce the number of microorganisms in the water to be discharged back into the environment for the later use of drinking, bathing, irrigation, etc. The effectiveness of disinfection depends on the quality of the water being treated (e.g., cloudiness, pH, etc.), the type of disinfection being used, the disinfectant dosage (concentration and time), and other environmental variables. Cloudy water will be treated less successfully, since solid matter can shield organisms, especially from ultraviolet light or if contact times are low. Generally, short contact times, low doses and high flows all militate against effective disinfection. Common methods of disinfection include ozone, chlorine, ultraviolet light, or sodium hypochlorite. Chloramine, which is used for drinking water, is not used in the treatment of waste water because of its persistence. After multiple steps of disinfection, the treated water is ready to be released back into the water cycle by means of the nearest body of water or agriculture. Afterwards, the water can be transferred to reserves for everyday human uses.

Chlorination remains the most common form of waste water disinfection in North America due to its low cost and long-term history of effectiveness. One disadvantage is that chlorination of residual organic material can generate chlorinated-organic compounds that may be carcinogenic or harmful to the environment. Residual chlorine or chloramines may also be capable of chlorinating organic material in the natural aquatic environment. Further, because residual chlorine is toxic to aquatic species, the treated effluent must also be chemically dechlorinated, adding to the complexity and cost of treatment.

Ultraviolet (UV) light can be used instead of chlorine, iodine, or other chemicals. Because no chemicals are used, the treated water has no adverse effect on organisms that later consume it, as may be the case with other methods. UV radiation causes damage to the genetic structure of bacteria, viruses, and other pathogens, making them incapable of reproduction. The key disadvantages of UV disinfection are the need for frequent lamp maintenance and replacement and the need for a highly treated effluent to ensure that the target microorganisms

are not shielded from the UV radiation (i.e., any solids present in the treated effluent may protect microorganisms from the UV light). In the United Kingdom, UV light is becoming the most common means of disinfection because of the concerns about the impacts of chlorine in chlorinating residual organics in the wastewater and in chlorinating organics in the receiving water. Some sewage treatment systems in Canada and the US also use UV light for their effluent water disinfection.

Ozone (O3) is generated by passing oxygen (O2) through a high voltage potential resulting in a third oxygen atom becoming attached and forming O3. Ozone is very unstable and reactive and oxidizes most organic material it comes in contact with, thereby destroying many pathogenic microorganisms. Ozone is considered to be safer than chlorine because, unlike chlorine which has to be stored on site (highly poisonous in the event of an accidental release), ozone is generated onsite as needed. Ozonation also produces fewer disinfection by-products than chlorination. A disadvantage of ozone disinfection is the high cost of the ozone generation equipment and the requirements for special operators.

Benefits of sewage treatment:

Save money by recycling a portion of waste water for use around garden.

Limit the impact of house waste on environment by becoming more self sufficient.

Protects precious source of ground water and saves rainwater in dams by recycling.

- Reduce impact on municipal sewage system by installing domestic treatment system, particularly grey water treatment system.

POSSIBLE QUESTIONS
UNIT-I
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Write an account of water born pathogen ?
2. Give the steps of typical sewage treatment plant
3. What is biochemical oxygen demand (BOD)?
4. What is a trickling filter?
5. What is MPN?

PART-C (8 MARKS)

1. Explain the water pollution
2. Write the detailed notes about the bacterial examination of water?
3. Explain the physical, chemical and biological methods of Sewage treatment
4. Give a detailed note on characters of sewage
5. Giv a notes on sludge digestion; activated sludge, aerating filters, oxidation pond

S.No	Unit I	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	_____ filters provide a high surface area to grow a biomass	Carbon	Diatom	Aerated	Micro	Aerated
2	_____ is a semiconductor photo catalyst.	Sodium hypochloride solution	Resorcinol	Triethylene glycol	Titanium dioxide	Titanium dioxide
3	_____ virus causes whooping cough.	*Varicella*	*Influenza*	*Bordetella pertussis*	*Rubella*	*Bordetella pertussis*
4	_____ is a semiconductor photo catalyst.	Sodium hypochloride solution	Resorcinol	Triethylene glycol	Titanium dioxide	Titanium dioxide
5	_____are relatively more abundant than the vegetative cells in the air	Spores	Infectious dust	Aerosols	Droplets	Spores
6	_____is an occupational disease	Brucellosis	Pulmanory disease	Pneumonitis	Meningitis	Brucellosis
7	_____can be a source of infectious diseases	Droplets	Aerosols	Dust	Flocs	Aerosols
8	Air borne infections are transmitted mainly by	Aerobes from person to person	Inhaling spores or hyphal fragments from soil or dead vegetation	Drinking contaminated water	Objects such as handkerchiefs that are contaminated with respiratory secretations	Aerobes from person to person
9	Air doesn't have a_flora	Indigenous	Auctothonous	Normal	None of the above	Normal
10	Airborne particles are a major cause of _____ allergies in humans.	Gastrointestinal	Urinary tract	Respiratory	Eye	Respiratory
11	Average salinity of seawater	15 ppt	25 ppt	35 ppt	45 ppt	35 ppt
12	Bacteriological examination of water usually employs	Total count	Multiple tube method	Membrane filters count	Plate count	Multiple tube method
13	*Chlorella pyrenoidosa is usually found in*	Activated	Sludge compost	Trickling filter	Oxidation	Activated

		sludge process			pond	sludge process
14	Copper is used in water treatement as a	Disinfectant	Indicator	Coagulant	Flocculants	Disinfectant
15	Droplet nuclei are significant in the transmission of diseases of the	Digestive system	Nervous system	Reproductive system	Respiratory system	Respiratory system
16	Ecological region at the lowest level of a body of water such as an ocean or a lake	Limnetic zone	Littoral zone	Profoundal zone	Benthic zone	Benthic zone
17	Effective air sanitizing is done by	Gamma radiation	UV radiation	Beta radiation	Gamma radiation	UV radiation
18	Elemental sulphur to sulphuric acid oxidised by ----------	Algae	Bacteria	Fungi	Viruses	Bacteria
19	Farmer's lung caused by exposure to spores of thermophilic _____	Fungi	Bacteria	Actinomycetes	Viruses	Actinomycetes
20	Fomites are	Insect vectors	Inanimate objects	Animate objects	Biological vectors	Inanimate objects
21	Formation of ---------- is crucial step in anaerobic digestion	Hydrogen	Carbondioxide	Water	Acetate	Acetate
22	HEPA filters are typically rated as _____ effective in removing dust.	90	99.92%	99.97%	90.99%	99.97%
23	In a lake the combined littoral and limnetic zone is known as	Profundal zone	Euphotic zone	Metalimnon	Epilimnon	Epilimnon
24	In Lemon sampler air is drawn at the rate of_____per minute and dispersed through the broth	20-25litre	25-30litre	30-35litre	35-40litre	20-25litre
25	Laminar airflow developed by_____.	Whittaker	Whitifield	Tyndall	Koch	Whitifield
26	Lilton large volume air sampler is an example for _____.	Sieve sampler	Impingement	Electrostatic precipitation	Centrifugal action	Electrostatic precipitation
27	Main photosynthetic body of the lake	Littoral zone	Limnetic zone	Profoundal zone	Paleic zone	Limnetic zone
28	Marine ecosystems cover approximately_____of the Earth's surface	7%	71%	17%	77%	71%
29	Microbes in air can be enumerated by	Settle plate method	Pour plate mehod	Spread plate method	Streak plate method	Settle plate method

30	Microorganisms found attached to the rock surface are reffered to as	Epipeplon	Episammon	Epixylon	Epilithon	Epipeplon
31	Of the different atmospheric layers_____is characterized by a heavy load of microorganisms	Troposphere	Stratosphere	Lithosphere	Atmosphere	Troposphere
32	Profoundal zone is	Open surface of water body	Sub-surface zone	Deepest zone	Side zone	Deepest zone
33	Relative humidity for survival of the microorganism is between ----------	25-45 percent	40-80 percent	40-60 percent	50-70 percent	40-80 percent
34	Schmutzdecke is a hypogeal biological layer formed on surface of slow sand filter by	Fungi	Bacteria	Protozoa	Algae	Bacteria
35	Slit sampler can collect upto_____% of the water droplet particles sprayed into air	85%	100%	95%	75%	85%
36	Sludge conditioning is accomplished by which of the following	Thickening	Elutriation	Chemical conditioning	Diluting with water	Thickening
37	Spores of_____travel over a thousand kilometers.	*Clostridium perfringens*	*Puccinia graminis*	*Sarcina lutea*	*Micrococcus luteus*	*Clostridium perfringens*
38	The amount of carbondioxide present in the atmosphere is near to	0.02%	0.03%	0.04%	0.05%	0.04%
39	The dominant genera of common saprophytic fungi in indoor air is	Aspergillus	Fusarium	Penicillium	Mucor	Penicillium
40	The filtering medium of the tank becomes coated with a microbial flora, the_____film	Biofilm	Zoogloeal film	.Neustonic	Algal bloom	Zoogloeal film
41	The most commonly efficient substrate used as a carbon source indenitrification during sewage treatement is	Methanol	Oxygen	Glucose	Sucrose	Methanol
42	The optimum rate of relative humidity for the survival of the most microorganisms is	40-80%	60-80%	50-80%	30-80%	40-80%
43	Viruses survive in the atmosphere at low temperature from	8 to 32°C	7 to 24°C	6 to 18°C	2 to 6°C	6 to 18°C
44	Which of the following can be seen in marine environment?	Halophiles	Barophiles	Psychrophiles	Hydrophiles	Halophiles

45	Which of the following is not an aerobic process?	Activated sludge process	Sludge digestion	Trickling filter	Oxidation pond	Trickling filter
46	Which of the following is not common in marine environment?	Luminous bacteria	Psychrophilic bacteria	Thermophilic bacteria	Barophilic bacteria	Luminous bacteria
47	Zone near shore area where sunlight penetrates the sediment and allows aquatic plants to grow	Littoral zone	Limnetic zone	Paleic zone	Profoundal zone	Littoral zone
48	Zoogloeal film formed in the trickling filter consists of	Bacteria	Algae	Protozoa	Algal bloom	Bacteria
49	Which of the following is the type of endosymbiosis	Commensalisms	cooperation	mutualism	predation	Commensalism
50	hich of the following microorganism grows well at temperatures above boiling point of water	Halobacterium	Methanococcus jannaschii	Pyrococcus furiosis	Bacillus	Pyrococcus furiosis
51	Which of the following method is used for removal of suspended materials from waste water	Filration	Purification	sedimentation	settlement	Sedimentation
52	Most of the indicator oranisms for detection of disease occurrence level in drinking water belongs to which of the following microroganisms group	Actinobacteria	Bacilli	Coliform	Firmicutes	Coliform
53	Which among the following microbes is not a prime concern for deterioration of water quality in drinking water	Legionella	Shigella	Vibrio parahaemolyticus	Vibrio vulnificus	Shigella
54	Which of the following test is used as preusmptive test for enumeration of coliform in water samples?	Most probable number	Heterocoliformcount	Aerobic colony count	colony forming unit	Most probable number
55	Which of the following promotes the biological transformation of dissolved organic matter to microbial biomass and carbondioxide	Primary	Secondary	Tertiary	Quaternary	Secondary
56	Inorganic nutrients are removed by biological means refer as which of the following treatment process	Primary	Secondary	Tertiary	Quaternary	Tertiary
57	In industrial processing plants, which of the following is the principle factor of treating waste water	Removal of microbes	Removal of organics	Removal of solids	Removal of liquids	Removal of organics
58	Along with inorganic and organic nutrients which of the following compounds are removed through tertiary treatment process	Heavy and trace metals	Lignocellulosic	Suspended matters	Floating materials	Heavy and trace metals

59	Zone near shore area where sunlight penetrates the sediment and allows aquatic plants to grow	Littoral zone	Limnetic zone	Paleic zone	Profoundal zone	Littoral zone
60	Microbes in air can be enumerated by	Settle plate method	Pour plate mehod	Spread plate method	Streak plate method	Settle plate method

MICROBIAL CONTAMINATION OF AIR:

Fungi and bacteria may cause biological deterioration of materials, and there are many microbial taxa responsible for biodeterioration. Fungi cause great damage to cultural heritage because they possess high biodeteriogenic capacity of organic matter (Borrego and Perdomo, 2012). May et al. (1993) listed dematiaceae fungi among the major agents of biodeterioration of surfaces and dark spots. Urzi et al. (2001) concluded that once fungi settle on and colonize surfaces, they are responsible for a great variety of alternation like black paints, intergranular growth, sugaring and biopitting. Species of Trichoderma, Penicillium, Botrytis, Trichothecium, Phoma, Chaetomium, Aspergillus, Cladosporium, Stemphylium, Alternaria, Hormodendrum, Aureobasidium, Papularia, Bacillus, Cellulomonas, Cellfalciculata, Cellvibrio, Sporocytophaga and Streptomyces have celluolytic properties, Aureobasidium, Chaetomium, Cladosporium, Botrytis, Trichoderma, Verticillium, Mucor, Epicoccum, Gymnoascus and Actinomycetes have proteolytic properties, all the proteolytic fungi listed above and Paecilomyces have lipolytic properties (Borrego et al., 2010 and Berent et al., 2011).

Inhalation of microorganisms may cause health threats. Wiszniewska et al. (2009) identified allergy to fungi in 31% of staff working at the national museum in Warsaw. Mycotoxins producing fungi, e.g. Aspergillus, Penicillium, and Stachybotrytis have been isolated from museum, libraries and archive settings (Eduard, 2009 and Skóra et al., 2015).

The evaluation of microorganisms in air and surface dust is the first step to control library's environment and maintains deterioration. The present study aims to evaluate microbiological indoor air quality at two libraries and their relationships with microclimatic factors and particulate matter (PM). Moreover the potentiality of fungi, particularly biodegradable taxa, with cellulolytic, proteolytic and lipolytic enzymatic activities was evaluated.

SOURCES OF CONTAMINATION

- Endotoxin or Lipopolysaccharide- allergens .
- Endotoxin is continually released from the cell wall of a gram negative bacteria.
- GRAM negative are more resistant against antibodies.
- These bacteria exist in soils and can be aerosolized- cause fever, shock and asthma
- MYCOTOXIN-air born spores.
- Natural and anthropogenic (human caused) air pollution consists of complex mixtures of chemical and biochemical species as well as pathogens, and the earth-sourced or earth-hosted component.
- Three major sources- 1. microbial decomposition of various substrates - cause pneumonitis.
- 2. associated with certain types of environments - Legionnaires' bacteria in water supplies.
- 3. stemming from infective individuals harboring a particular pathogen-cause tuberculosis.

TRANSPORT OF AIRBORNE PATHOGENS

- **Direct or indirect methods**
- **fungal spores released by soil fungi.**
- **People can be affected basically by any naturally occurring or human caused process that kicks up dust or other small particles that might blow in the wind.**
- **Bioaerosols are associated with soil or vegetation because the microorganisms are usually transported by the aerosol, or in other words, the soil particle.**
- **This process require dry conditions.**

BIOAVAILABILITY

- *Outdoor concentrations of airborne bacteria generally were higher than those indoors but similar in summer and winter.*
- *Bacterial concentrations indoors showed more seasonal difference, which may be due to changes in occupant dress and activities as well as ventilation patterns during the cooling and heating seasons.*

IMPACT ON HUMAN HEALTH

- Naturally occurring or human caused airborne substances Allergies- affect roughly 17% of the U.S. population (*Earth Materials and Health,* pg.61)
- Psittacosis- dried bird droppings from infected birds, blown into air
- Legionnaire's disease- droplets from air-conditioning systems, water tanks or where any bacterium grows
- Aspergillosis- Fungal spores inhaled from decomposing organic matter

AIR BORN VIRAL DISEASE

- Chickenpox- causes blister-like rash
- Flu/influenza- respiratory illness
- Measles- also known as rubeola, rash and fever
- Rubella/German measles- rash and fever
- Mumps- fever and swelling of salivary glands
- Smallpox- extensive rash and high fever
- Hantavirus pulmonary syndrome- respiratory disease from contact with infected rodents
- Pleurodynia- chest wall pain, gastrointestinal/respiratory illness
- Common cold
- Severe Acute Respiratory Syndrome (SARS)- identified as a new disease in 2003

AIR BORN BACTERIAL DISEASE

- Whooping cough- severe coughing fits

- Meningitis- fever, rash, nausea
- Diphtheria- nerve and organ damage
- Pneumonia- spectrum of illness ranges from asymptomatic infection to severe disease
- Tuberculosis- can be latent, weight loss, fever, chest pain.
- Pulmonary anthrax- caused by handling products from infected animals or by breathing in the anthrax spores, spreads from person to person
- Staphylococcus respiratory infection, sepsis, other cutaneous infections- about 30% of people carry this in their nose
- Streptococcus respiratory infection- an example is strep throat
- Legionellosis- fever, chills, cough, can be treated with antibiotics
- Pneumonic plague, bubonic plague- usually transmitted to humans by infected rodent flea bites

MICROBIAL INDICATOR OF AIR(MICROORGANISMS AS BIOLOGICAL INDICATORS OF AIR POLLUTION S. Waldner-Sander, K. Botzenhart EXS 51: Advances in Aerobiology ©1987 Birkhauser Verlag Basel)

Many countries have recently established networks for monitoring air pollution, which generally employ physicochemical methods of analysis. In many places, biological indicator systems are also used to evaluate air quality. Some highly standardized methods employ lichens or tobacco, which react selectively with particulate air pollutants (ARNDT et al., 1985), or cultures of grass which accumulate heavy metals (VOl, German Assoc. of Engineers, 1978). Frequently a combination of different species of green plants and lichens is used to detect the effects of various air pollutants. Such bioindicators, being living organisms, react to a sum total of toxic effects in the air and thus may yield valuable information more relevant to the health of man than that provided merely by physicochemical measurement of air pollutants. Bacteria, the smallest organisms with a complete metabolism, have long been used to assess the efficacy of antimicrobial agents or equipment and have recently been shown to be very convenient indicators of DNA-damaging substances, e.g., in conjunction with the Ames test (AMES et al., 1975). In 1968, DRUETT and PACKMAN demonstrated an adverse effect of unknown air constituents on the viability of bacteria. This effect was confirmed by DRUETT and MAY (1968) and was later termed "open air factor" (OAF). DEMIK (1976) showed a correlation between OAF concentration, on the one hand, and the concentration of ozone and motor vehicle pollution, on the other hand; he also documented the damaging effect of ozonized cyclohexane on bacterial DNA. Many other ozonized hydrocarbons have been found to be germicidal and mutagenic in smog chamber experiments; toxic effects on microorganisms under such conditions can be documented by changes in the death rate calculated from numbers of colony-forming units (CFU) detected over a given period of time (NOVER and BOTZENHART, 1983, 1985).

Prior to exposure of the test strains to open air at a given site, two sampling chambers were prepared as follows: Membrane filters were loaded with 100-200 cells of a given test strain by adsorption of cells removed from the culture medium during the exponential growth phase. 30 filters of each strain were placed in the incubation chambers. Subsequently, the chambers were purged with synthetic air, adjusted to the desired relative humidityusing saturated salt solution, sealed, and transported to the exposure site. After a minimum exposure time of 30 min, transfer of the membrane filters of one chamber to agar plates was initiated and continued at regular intervals up to a maximum exposure time of 90 min. Immediately thereafter, filters which served as controls were removed from the second chamber which remained sealed up to this time. After 1 or 2 days incubation, colonies were counted and the

reduction rate ~ was calculated from the difference between control and exposure diagrams. The test strains were exposed at 3 field stations in the state of Baden WUrttemberg, Federal Republic of Germany (FRG) which are equipped with extensive instrumentation for monitoring meteorlogical parameters and levels of: sulfur dioxide, nitrous oxides, ozone, and carbon monoxide. One station is located in an elevated region of the Black Forest near the K8lbelescheuer. At this site, immission levels of SO? and NO x are generally very low, those of ozone high. The other sites are sItuated rn 2 forest areas near Stuttgart: Welzheimer Wald and Schonbuch. In the Welzheimer Wald, NO X and S~? are detected sporadically and ozone levels correlate with levels in other west German regions of similar altitude. In the Schonbuch; the NO X' S02' CO and 03 levels are affected by nearby motor vehicle traffic.

The feasibility of employment of bacteria, yeasts and molds as bioindicators of air pollution was tested in preliminary experiments under field conditions. Spores of fungi and Bacillus subtilis were readily excluded due to their stability during the given exposure period. Considerably higher sensitivity to the open air was revealed by the yeast Candida albicans and the bacteria: Staphylococcus epidermidis, Serratia marcescens and Micrococcus luteus. Serratia marcescens proved to be unstable, however. A pronounced loss of viability during transport and under test conditions caused impaired reproducibility of results and thus the applicability of this bacteria as an indicator of air pollution was limited.

ENUMERATION OF MICROORGANISMS IN AIR, CONTROL OF AIRBORNE MICROORGANISM (http://ecoursesonline.iasri.res.in/mod/page/view.php?id=5231)

29.1 Enumeration of Microorganisms in Air

Various methods commonly applied for enumeration and detection of microorganisms can be subdivided into:

- Microscopic methods
- Culture methods
- Combination of both

29.2 Microscopic Methods

These consist of

- Letting air through a membrane filter or placing a glass coated with a sticky substance (e.g. vaseline), in the path of air
- Staining of the trapped microorganisms and
- Microscopic testing consisting of cell counting

Staining with acridine orange and examination under a fluorescence microscope is often applied. The final result is given as a total number of microbes in 1 m^3 of air. The advantage of this method is that it allows the detection of live and dead microbes in air, as well as those, which do not abundantly flourish in culture media. Due to this, the number of microbes determined is usually higher by one order of magnitude than in culture methods. In addition, it is possible to detect and identify other biological agents e.g. plant pollen, allergenic mites, abiotic organic dust (fragments of skin, feathers, plants, etc.).

However the methods have a serious drawback: inability to determine the species of microbes (bacteria, fungi, viruses).

29.3 Culture Methods

These methods consist of transferring microbes from air onto the surface of the appropriate culture medium. After a period of incubation at optimal temperature, the formed colonies are counted and the result is given as cfu/m^3 of air (colony forming units). Because a colony can form not only from a single cell, but also from a cluster of cells, the air may contain more microbes than suggested by the CFU result. Besides, the method allows the detection of only the

cells that are viable and those which are able to grow upon the medium used. Microbes transferred to the culture medium require resuscitation as they were subjected to the influence of unfavourable conditions. Therefore it is recommended to supplement the culture mediums are required to be supplemented with components such as betaine and catalase. Betaine, the methylic derivative of the glycine amino acid, is utilized by bacteria to maintain osmotic balance, and as a donor of methylic groups it is essential during the processes of biosynthesis. Catalase however breaks down harmful peroxides created in air as a result of UV radiation.

However, testing of viruses differs significantly from the methods utilized for other organisms because:

- They may develop only in living cells, therefore they require tissue cultures (e.g. the epithelium of human trachea or monkey's kidney) or, in the case of bacteriophages, bacterial cultures,
- Species identification of detected viruses is meticulous and, among other things, consists of performing electrophoresis or utilizing antiserum that contains antibodies of common viruses,
- Drawing large quantities of air is essential (over 1000 dm^3, at least one order of magnitude higher than in the case of bacteria), as the amount of viruses in air is rather small (this especially concerns the enteroviruses).

After transferring the viruses onto the surface of a single-layer culture, the viruses penetrate the cells, reproduce in them, and after their destruction attack the neighboring cells. Consequently, the areas around the initial places of the cell infections get cleared of cells – this clearing is called plaques. Therefore, the number of viruses detected is given as the number of units that form the plaques, in short pfu/m^3 (plaque forming units). It has to be pointed out though, that such a method only allows the detection of viruses capable of infecting the utilized cells.

29.4 Sampling of Air

There are four basic ways of sampling the air for use in culture methods:

- Koch's sedimentation method
- Filtration method (also used in microscopic methods)
- Centrifugation
- Impact methods

29.5 Sedimentation Method

This 'Settling Plate Technique' based on this approach is the simplest and is often used by air microbiologists. The principle behind this method is that the bacteria carrying particles are allowed to settle onto the medium for a given period of time and incubated at the required temperature. A count of colonies formed shows the number of settled bacteria containing particles. In this method petridishes containing an agar medium of known surface area are selected so that the agar surface is dry without any moisture. Choice of the medium depends upon the kind of microorganisms to be enumerated. For an overall count of pathogenic, commensal and saprophytic bacteria in air blood agar can be used. For detecting a particular pathogen which may be present in only small numbers, an appropriate selective medium may be used. Malt extract agar can be used for molds. The plates are labeled appropriately about the place and time of sampling, duration of exposure etc. Then the plates are uncovered in the selected position for the required period of time. A Petri dish containing agar medium is kept covered and, at the time of sampling, the cover is removed from the Petri dish so that the agar surfaces is exposed to air for a few minutes. The Petri dish is now incubated. One can see a certain number of colonies developing on agar medium (Fig. 29.1). Each colony represents a particle carrying microorganisms which has fallen on the agar surface. The optimal duration of exposure should give a significant and readily countable number of well isolated colonies, for example about 30-100 colonies. Usually it depends on the dustiness of air being sampled. In occupied rooms and hospital wards the time would generally be between 10 to 60 m. During sampling it is better to keep the plates about I metre above the ground. Immediately after exposure for the given period of time, the plates are closed with the lids. Then the plates are incubated for 24 hrs at 37°C for aerobic bacteria and for 3 days at 22°C for saprophytic bacteria. For molds incubation temperature varies from 10-50°C for 1-2 weeks. After incubation the colonies on

each plate are counted and recorded as the number of bacteria carrying particles settling on a given area in a given period of time.

The use of settle plates is not recommended when sampling air for fungal spores, because single spores can remain suspended in air indefinitely. Settle plates have been used mainly to sample for particulates and bacteria either in research studies or during epidemiologic investigations. Results of sedimentation sampling are typically expressed as numbers of viable particles or viable bacteria per unit area per the duration of sampling time (i.e. CFU/area/time); this method can not quantify the volume of air sampled. Because the survival of microorganisms during air sampling is inversely proportional to the velocity at which the air is taken into the sampler, one advantage of using a settle plate is its reliance on gravity to bring organisms and particles into contact with its surface, thus enhancing the potential for optimal survival of collected organisms. This process, however, takes several hours to complete and may be impractical for some situations.

29.5.1 Limitation

Though the method has the advantage of simplicity, it has certain limits.

- In this method only the rate of deposition of large particles from the air, not the total number of bacteria carrying particles per volume, is measured.
- Growth of bacteria in the settled particles may be affected by the medium used since not all microorganisms are growing well on all media.
- Moreover since air currents and any temporary disturbances in the sampling area can affect the count, many plates have to be used.
- Since only particles of certain dimensions tend to settle on to the agar surface and, also, the volume of air entering inside the Petri dish is not known, this technique gives only a rough estimate and can be used only to isolate air-borne microorganisms.
- However, one can gather information about the kind of air-borne microbes occurring in a particular area by repeated use of settling plate technique for a fixed period of time.

29.6 Filtration Methods

The methods consist of using an aspirator to suck in a given volume of air, passing it through a sterile absorbing substance (liquid or solid) and transferring the filtered microbes onto the appropriate culture medium. After a pre-determined time of incubation the resulting colonies are counted. Most often, a membrane filter or a physiological solution (0.85% NaCl) is utilized for the filtration of air. Filtration using liquids (sometimes classified as the impact method) is one of the most often used and highly valued techniques of sampling bioaerosol (Fig. 29.2). It results in high output of microbe isolation as well as significant survival of the filtered microbes. The method may be utilized in virus testing as long as the remaining microbes are neutralized (e.g. with chloroform) and the liquid is concentrated before its introduction into the cell culture.

The filtration process through membrane filters allows the utilization of both culture methods (filters containing microbes are placed directly upon the culture media or are rinsed and then inoculated) as well as the microscopic methods (filters are stained and observed under a microscope).
These are simple methods for collecting particles from air. The filter can be made of any fibrous or granular material like sand, glass fibre and alginate wool (in phosphate buffer). However, recovery of organisms for culture is not so easy. The membrane filter devices are adaptable to direct collection of microorganisms by filtration of air. These methods are also rather inexpensive and not complicated; they possess two significant advantages over the sedimentation methods:

- The volume of the air tested is known,

- It is possible to detect the very small aerosol that creates the respiratory fraction (nevertheless it is still impossible to determine its size

29.6.1 Tube sampler

This is one of the oldest devices for collecting and enumerating microorganisms in the air. It consists of a tube with an inlet at the top and an outlet at the bottom which is narrower than the top end. Near the bottom there is a filter of wet sand which is supported by a cotton plug below. The entire device can be sterilized. After sterilization the air to be sampled is allowed to pass through the sand and cotton. Microorganisms as well as dust particles containing microorganisms in the air are deposited in the sand filter as the air passes through it. Later the sand is washed with broth and a plate count is made from the broth by taking aliquotes of the broth.

29.6.2 Millipore filter

This type of filters is made of pure and biologically inert cellulose ethers. They are prepared as thin porous, circular membranes of about 150 µm thickness. The filters have different porosity. The assemblage contains a funnel shaped inlet and a tube like outlet. In between these two the filter is fitted. The outlet may be connected to a vacuum pump to suck known amount of air. After collecting required volume of air through the filter, it can directly be placed onto the surface of a solid medium. After incubation colonies formed can be counted.

However, the disadvantage of this method is that it has a significantly low output as the process of passing the air through pores of the filter creates resistance. That's why the method is not recommended for microbe testing, but is routinely put to use in detection of endotoxins in air.

29.7 Centrifugation Methods

29.7.1 Air centrifuge

The first primitive type of air centrifuge was developed by Wells in 1993. The principle of air centrifuge is that the particles from air are centrifuged onto the culture medium. In his air centrifuge sampled air was passed along a tube which was rotated rapidly on its long axis. The inner surface of the tube was lined with culture medium and any bacteria containing particle deposited on it grew into a colony on incubation. A modern version of this centrifuge is the Reuter centrifugal air sampler, which is portable and battery powered. It resembles a large cylindrical torch with an open ended drum at one end. The drum encloses impeller blades which can be rotated by battery power when switched on. A plastic strip coated with culture medium can be inserted along the inner side of the drum. Air is drawn into the drum and subjected to centrifugal acceleration. This causes the suspended particles to impact on the culture medium. After sampling the strip is removed from the instrument and incubated at 37°C for 48 h. Later the colonies can be counted. Advantage of this sampler is that it is very convenient for transportation and use. However, the disadvantage is that it is less efficient than the slit sampler in detecting particle below 5 mm in diameter. More over the size of the air being sampled cannot be accurately controlled.

29.7.2 Impact methods

These methods consist of using an aspirator to suck in a pre-determined amount (volume) of air, which collides with the nutrient agar at high speed. It causes the microbes in the air to stick to the surface, which after a specific time of incubation, form colonies. The impact methods are the most highly valued and most often used methods of detecting microbes in air. Their biggest advantage is the possibility of detecting and determining the respiratory fraction of the bioaerosol, in other words, determining the size distribution of its particles. The methods can be utilized to test viruses (trapped microbes are swept from the surface of the culture medium and, after the elimination of other microbes with chloroform, introduced into the cell culture).

A disadvantage for the impact method is a decline in the microbes viability caused by the shock of a sudden collision with nutrient agar and also a possibility of the nutrient culture getting overgrown in cases of high air pollution. The above stated methods are usually not cheap. The most widely known device that is based on the impact technique is the Andersen's apparatus, in which the air is drawn in passes through six vertically positioned sieves. A petri dish with nutrient agar is placed underneath each sieve. The speed of the passing air increases as it passes through the consecutive sieves, consequently causing greater impact force as it collides with the sieves. As a result, the heaviest (largest) particles settle upon the first sieve, whereas the lighter (smaller) ones are drawn in by the current of the passing air. As they pass through the consecutive sieves, the increasingly smaller and faster particles collide with the nutrient agar. Consequently the particles of the biological aerosol are sorted according to their size and the colonies are then derived from particles of particular size. This way, by counting the colonies upon the consecutive plates, it is possible to determine the ratio of particles which settle in the upper (higher positioned plates) and lower respiratory system (lower plates).

29.7.2.1 Sampling of measured volume of air

An improvised method wherein a measured volume of air is sampled has also been developed (Fig. 29.3). These are sieve and slit type devices. A sieve device has a large number of small holes in a metal cover, under which is located a petridish containing an agar medium. A measured volume of air is drawn, through these small holes. Airborne particles impinge upon the agar surface. The plates are incubated and the colonies counted. In a slit device the air is drawn through a very narrow slit onto a petridish containing agar medium. The slit is approximately the length of the petridish. The petridish is rotated at a particular speed under the slit. One complete turn is made during the sampling operation

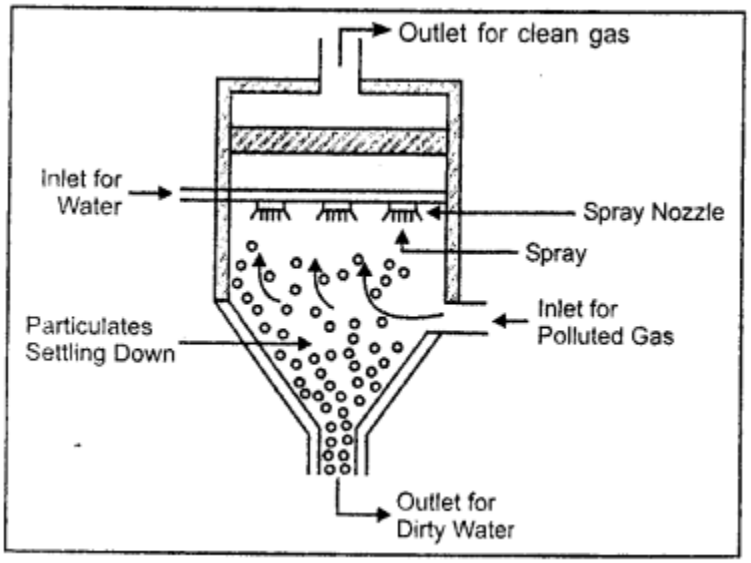
Fig. 29.3 The sieve-impaction sampling method

29.7.2.2 Selection of air sampler

The following factors must be considered when choosing an air sampling instrument:

- Viability and type of the organism to be sampled
- Compatibility with the selected method of analysis
- Sensitivity of particles to sampling
- Assumed concentrations and particle size
- Whether airborne clumps must be broken (i.e. total viable organism count vs. particle count)
- Volume of air to be sampled and length of time sampler is to be continuously operated
- Background contamination
- Ambient conditions
- Sampler collection efficiency
- Effort and skill required to operate sampler
- Availability and cost of sampler, plus back-up samplers in case of equipment malfunction
- Availability of auxiliary equipment and utilities (e.g. vacuum pumps, electricity, and water)

AIR SANITATION



Scrubber

Effects on cardiovascular health: exposure is a risk factor correlating with increased total mortality from cardiovascular events (range: 12% to 14% per a 10 microg/m for stroke, particularly in developing countries where pollutant levels are highest. A 2007 study found that in women air pollution is associated not with hemorrhagic but with ischemic stroke. Air pollution was also found to be associated with increased incidence and mortality from coronary stroke in a cohort study in 2011. Associations are believed to be causal and effects may be mediated by vasoconstriction, low imbalance or other mechanisms. Effects on cystic fibrosis: A study from around the years of 1999 to 2000, by the University of Washington, showed that patients near and around particulates air pollution had an increased risk of pulmonary exacerbations and decrease in lung function. Patients were examined before study for amounts of specific pollutants like cenocepacia as well as their socioeconomic standing. Participants involved in the study were located in the United States in +close needed] During the time of the study in the study lived in or near large metropolitan areas in order to be close to same patients had higher level of pollutants found in their system because of more emissions in larger cities. As cystic fibrosis patients already suffer from decreased lung function, everyday pollutants such as smoke, emissions from au indoor heating devices could further compromise lung function. Effects on COPD and asthma: diseases such as chronic bronchitis risk of developing asthma and COPD from increased exposure to t Additionally, air pollution has been associated with increased hospitalizations and mortality from asthma and COPD. A study conducted in 1960 compared 293 London residents with 477 residents of Gloucester, Peterborough, and Norwich, three towns with low reported death rates from chronic bronchitis. All subjects were male postal truck drivers aged 40 to 59. Compared to the subject subjects exhibited more severe respiratory symptoms (including cough, phlegm, and dyspnea), reduced lung function (FEV1 purulence. The differences were more pronounced for subjects aged 50 to 59. The study controlled for age and smoking habits, so concluded that air pollution was the most likely cause of the observed differences. It is believed that much like environment serious health hazards become more apparent. Studies have shown that in urban areas patients suffer mucus hypersecretion, lower levels of lung function, and more self diagnosis of chronic bronchitis and emphysema A 2007 review of evidence found ambient air pollution exposure is a risk factor correlating with increased total mortality from cardiovascular events (range: 12% to 14% per a 10 microg/m3 increase). Air pollution is also emerging as a risk factor eloping countries where pollutant levels are highest. A 2007 study found that in women air pollution is associated not with hemorrhagic but with ischemic stroke. Air pollution was also found to be associated with increased incidence and mortality from nary stroke in a cohort study in 2011. Associations are believed to be causal and effects may be mediated by vasoconstriction, low-grade inflammation or autonomic nervous system : A study from around the years of 1999 to 2000, by the University of Washington, showed that patients near and around particulates air pollution had an increased risk of pulmonary exacerbations and decrease in lung function. Patients were examined before study for amounts of specific pollutants like Pseudomonas aeruginosa as well as their socioeconomic standing. Participants involved in the study were close proximity to an Environmental Protection

study 117 deaths were associated with air pollution. in the study lived in or near large metropolitan areas in order to be close to medical help. These same patients had higher level of pollutants found in their system because of more emissions in larger cities. As cystic fibrosis patients already suffer from decreased lung function, everyday pollutants such as smoke, emissions from automobiles, tobacco smoke and improper use of indoor heating devices could further compromise lung function. Effects on COPD and asthma: Chronic obstructive pulmonary disease (COPD) includes chronic bronchitis and emphysema. Researchers have demonstrated increased risk of developing asthma and COPD from increased exposure to traffic-related air pollution. Additionally, air pollution has been associated with increased hospitalizations and mortality from asthma and COPD. A study conducted in 1960-1961 in the wake of the Great Smog compared 293 London residents with 477 residents of Gloucester, Peterborough, and Norwich, three towns with low reported death rates from chronic bronchitis. All subjects were male postal truck drivers aged 40 to 59. Compared to the subjects from the outlying towns, the London subjects exhibited more severe respiratory symptoms (including cough, phlegm, and dyspnea), and peak flow rate), and increased sputum production and purulence. The differences were more pronounced for subjects aged 50 to 59. The study controlled for age and smoking habits, so concluded that air pollution was the most likely cause differences. It is believed that much like cystic fibrosis, by living in a more urban environment serious health hazards become more apparent. Studies have shown that in urban hypersecretion, lower levels of lung function, and more self diagnosis of chronic bronchitis and emphysema found ambient air pollution exposure is a risk factor correlating with increased total mortality from cardiovascular events increase).

Air pollution is also emerging as a risk factor eloping countries where pollutant levels are highest. A 2007 study found that in women air pollution is associated not with hemorrhagic but with ischemic stroke. Air pollution was also found to be associated with increased incidence and mortality from nary stroke in a cohort study in 2011. Associations are believed to be causal and effects may grade inflammation or autonomic nervous system : A study from around the years of 1999 to 2000, by the University of Washington, showed that patients near and around particulates air pollution had an increased risk of pulmonary exacerbations and decrease in lung function. Patients were examined before the Pseudomonas aeruginosa or Burkholderia as well as their socioeconomic standing. Participants involved in the study were Agency. [clarification pollution. Many patients medical help. These same patients had higher level of pollutants found in their system because of more emissions in larger cities. As cystic fibrosis patients already suffer from decreased lung function, everyday tomobiles, tobacco smoke and improper use of Chronic obstructive pulmonary disease (COPD) includes . Researchers have demonstrated increased related air pollution. Additionally, air pollution has been associated with increased hospitalizations and mortality from at Smog of 1952 compared 293 London residents with 477 residents of Gloucester, Peterborough, and Norwich, three towns with low reported death rates from chronic bronchitis. All subjects were male postal s from the outlying towns, the London subjects exhibited more severe respiratory symptoms (including cough, phlegm, and dyspnea), and peak flow rate), and increased sputum production and purulence. The differences were more pronounced for subjects aged 50 to 59. The study controlled for age and smoking habits, so concluded that air pollution was the most likely cause , by living in a more urban environment serious health hazards become more apparent. Studies have shown that in urban hypersecretion, lower levels of lung function, and more self

The following items are commonly used as pollution control devices by industry or transportation devices. They can either destroy stream before it is emitted into the Particulate control 1. Mechanical collectors (dust cyclones 2. Electrostatic precipitators is a particulate collection device that removes particles from a flowing gas (such as air) using the force of an induced electrostatic charge. Electrostatic precipitators are highly efficient filtration devices that minimally impede the flow of gases through the device, and can easily remove fine particulates such as dust and smoke from the air 3. Baghouses - Designed to handle heavy dust loads, a dust collector consists of a blower, dust filter, a filter-cleaning system, and a dust receptacle or dust removal system (distinguished from air cleaners which utilize disposable filters to remove the dust). Particulate scrubbers Wet scrubber is a form of pollution control technology.

The term describes a variety of devices that use pollutants from a furnace flue gas or from other gas streams. In a wet scrubber scrubbing liquid, by spraying it with the liquid, by forcing it through a pool of liquid, or by some other contact method, so as to remove the Scrubbers Baffle spray scrubber Cyclonic spray scrubber Ejector venturi scrubber Mechanically aided Spray tower Wet scrubber NOx control Low NOx burners Selective are commonly used as pollution control devices by industry or transportation

devices. They can either destroy contaminants or remove them from an exhaust stream before it is emitted into the atmosphere. dust cyclones, multicyclones) Electrostatic precipitators- An electrostatic precipitator (ESP), or electrostatic air cleaner is a particulate collection device that removes particles from a flowing gas (such as air) using the force of an induced electrostatic charge. Electrostatic precipitators are highly ration devices that minimally impede the flow of gases through the device, and can easily remove fine particulates such as dust and smoke from the air Designed to handle heavy dust loads, a dust collector consists of a blower, cleaning system, and a dust receptacle or dust removal system (distinguished from air cleaners which utilize disposable filters to remove the dust). Wet scrubber is a form of pollution control technology. The term describes a variety of devices that use pollutants from a furnace flue gas or from other gas streams. In a wet scrubber, the polluted gas stream is brought into contact with the scrubbing liquid, by spraying it with the liquid, by forcing it through a pool of liquid, or by some other contact method, so as to remove the pollutants. scrubber scrubber scrubber aided scrubber burners Selective catalytic reduction.

Electrostatic precipitators are highly ration devices that minimally impede the flow of gases through the device, and can easily remove fine particulates such as dust and smoke from the air stream. Designed to handle heavy dust loads, a dust collector consists of a blower, cleaning system, and a dust receptacle or dust removal system (distinguished from air cleaners which utilize disposable filters to remove the dust). Wet scrubber is a form of pollution control technology. The term describes a variety of devices that use pollutants from a furnace flue gas or from other gas , the polluted gas stream is brought into contact with the scrubbing liquid, by spraying it with the liquid, by forcing it through a pool of liquid, t of Selective non-catalytic NOx scrubbers Exhaust gas recirculation Catalytic converter VOC abatement Adsorption systems, Flares Thermal oxidizers Catalytic converters Biofilters Absorption (scrubbing) Cryogenic condensers Acid Gas/SO2 control Wet scrubbers Dry scrubbers Flue-gas desulfurization

Mercury control Sorbent Injection Electro-Catalytic Oxidation K-Fuel Atmospheric dispersion The basic technology for analyzing air pollution is through the use of a variety of models for predicting the transport of air pollutants in the lower atmosphere. catalytic reduction (SNCR) recirculation Catalytic converter (also for VOC control) Adsorption systems, such as activated carbon oxidizers converters (scrubbing) condensers desulfurization Technology Oxidation (ECO) The basic technology for analyzing air pollution is through the use of a variety of predicting the transport of air pollutants in the lower atmosphere. The principal methodologies are Point source dispersion, used for industrial sources. Line source dispersion, used for airport and Area source dispersion, u Photochemical models, used to analyze rea The point source problem is the best understood, since it involves simpler mathematics and has been studied for a long period of time, dating back to about the year 1900. It uses a Gaussian dispersion model for continuous buoyant pollution plumes to predict the air pollution isopleths, with consideration given to wind velocity, stack height, emission rate and stability class (a measure of atmospheric extensively validated and calibrated with experimental data for all sorts of atmospheric conditions. Visualization of a buoyant Gaussian air pollution dispersion plume as used in many atmospheric dispersion models. The roadway air dispersion model response to requirements of the Transportation (then known as the Federal Highway Administration) to understand impacts of proposed new highways upon air quality, especially active in this model development, among which were: the Environmental Research and Technology (ERT) group in in Sunnyvale, California dispersion, used for industrial sources. dispersion, used for airport and roadway air dispersion modeling dispersion, used for forest fires or dust storms models, used to analyze reactive pollutants that form smog The point source problem is the best understood, since it involves simpler mathematics and has been studied for a long period of time, dating back to about the year 1900. It uses dispersion model for continuous buoyant pollution plumes to predict the air , with consideration given to wind velocity, stack height, emission rate d stability class (a measure of atmospheric turbulence). This model has been extensively validated and calibrated with experimental data for all sorts of atmospheric a buoyant Gaussian air pollution dispersion plume as used in many atmospheric roadway air dispersion model was developed starting in the late 1950s and early response to requirements of the National Environmental Policy Act and the U.S. Departm (then known as the Federal Highway Administration) to understand impacts of proposed new highways upon air quality, especially in urban areas. Several research groups were active in this model development, among which were: the Environmental Research and

Technology (ERT) group in Lexington, Massachusetts, the ESL Inc. group and the California Air Resources Board group in Sacramento KARPAGAM ACADEMY OF HIGHER EDUCATION Environmental and Agricultural Microbiology 2018-2020 Page 32 modeling smog The point source problem is the best understood, since it involves simpler mathematics and has been studied for a long period of time, dating back to about the year 1900. It uses dispersion model for continuous buoyant pollution plumes to predict the air , with consideration given to wind velocity, stack height, emission rate). This model has been extensively validated and calibrated with experimental data for all sorts of atmospheric a buoyant Gaussian air pollution dispersion plume as used in many atmospheric was developed starting in the late 1950s and early 1960s in U.S. Department of (then known as the Federal Highway Administration) to understand impacts of earch groups were active in this model development, among which were: the Environmental Research and , the ESL Inc. group Sacramento,

The research of the ESL group received a boost with a contract award from United States Environmental Protection Agency to validate a line source model using as a tracer gas. This program was successful in validating the line source model developed by ESL Inc. Some of the earliest uses of the model were in court cases involving Arlington, Virginia portion of Interstate 66 and the widening project through East Brunswick, New Jersey. Area source models were developed in 1971 through 1974 by the ERT and ESL groups, but addressed a smaller fraction of total air pollution emissions, so that their use and need was not as widespread as the line source model, which enjoyed hundreds of different applications as early as the 1970s. Interest in the microbial biodegradation of pollutants has intensified in recent years as humanity ways to clean up contaminated environments. biotransformation methods endeavour to harness the aston naturally occurring ability of microbial xenobiotic metabolism to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polyaromatic hydrocarbons (PAHs), heterocyclic compounds), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in recent years have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally providing unprecedented insights into key biodegradative pathw the ability of organisms to adapt to changing environmental conditions. The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to pr a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing ic versatility of microorganisms to degrade or convert such compounds. New methodological breakthroughs in sequencing, genomics, proteomics, bioinformatics are producing vast amounts of information. In the field of Environmental microbiology based global studies open a new era providing unprecedented in silico views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to the molecular adaptation strategies to changing environmental conditions. Functional genomic and metagenomic approaches are increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds and they will certainly accelerate the development of bioremediation technologies processes. The research of the ESL group received a boost with a contract award from ne source model using sulfur as a tracer gas. This program was successful in validating the line source model developed by ESL Inc. Some of the earliest uses of the model were in court cases involving and the New Jersey Area source models were developed in 1971 through 1974 by the ERT and ESL groups, but addressed a smaller fraction of total air pollution emissions, so that their use and need was not as widespread as.

BIOREMEDIATION

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. Technologies c classified as in situ or ex situ. In situ at the site, while ex siti involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation related technologies, phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration and biostimulation. Bioremediation bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc., that help encourage the growth (i.e., bioavailability) of the pollution medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform t known as bioremediators. However, not all contaminants are easily treated by bioremediation using microorganisms. For example, absorbed or captured by microorganisms. A recent experiment, however, sugg have

some success absorbing lead from contaminated Bone char has been shown to bioremediate small amounts of Cadmium, Copper, and Zinc. The assimilation of metals such as Phytoremediation is useful in these circumstances because natural plants or able to bioaccumulate these toxins in their above removal. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use. Some damaged artifac could be specified as bio remediating agents. The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation organisms specifically designed for bioremediation has great potential. The Deinococcus radiodurans (the most consume and digest toluene and Bioremediation contaminated soil, aquifers, marine pollutants, air pollutants, stimulation of oil spills degradation. Bioremediation of air pollutants. Bioleaching – recovery of metal from ores testing for biodegradability Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. Technologies c In situ bioremediation involves treating the contaminated material lves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation related technologies, phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration . Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc., that help encourage the growth (i.e., bioavailability) of the pollution-eating microbes medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators. However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead absorbed or captured by microorganisms. A recent experiment, however, suggests that fish bones have some success absorbing lead from contaminated soil Bone char has been shown to bioremediate small amounts of Cadmium, Copper, and Zinc. The assimilation of metals such as mercury into the food chain may worsen matters. is useful in these circumstances because natural plants or transgenic these toxins in their above-ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use. Some damaged artifacts at museums contain microbes which could be specified as bio remediating agents. The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of latory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation biotransformation processes. The use of genetic engineering pecifically designed for bioremediation has great potential. The (the most radio resistant organism known) has been modified to and ionic mercury from highly radioactive nuclear waste. contaminated soil, aquifers, marine pollutants, air pollutants, stimulation of oil recovery of metal from ores – Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. Technologies can be generally bioremediation involves treating the contaminated material lves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation related technologies, phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc., eating microbes within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to he function of bioremediation are known as bioremediators. However, not all contaminants are easily treated by bioremediation lead are not readily ests that fish bones Bone char has been shown to bioremediate small amounts of Cadmium, Copper, and Zinc. The may worsen matters. transgenic plants are ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration ts at museums contain microbes which could be specified as bio remediating agents. The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation engineering to create pecifically designed for bioremediation has great potential. The bacterium organism known) has been modified to from highly radioactive nuclear waste.

Mycoremediation : Mycoremediation is a form of bioremediation in which decontaminate the area. The term fungal mycelia in bioremediation. One of the primary roles of the ecosystem is decomposition secretes extracellular enzymes and building blocks of plant fiber. These are organic compounds composed of long chains of carbon and

hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutan strains have been reported to successfully degrade the conducted experiment, a plot of soil contaminated with of oyster mushrooms; traditional bioremediation techniques (bacteria) were used on control plots. After four weeks, more than 95% of many of the PAH (had been reduced to non-toxic components in the mycelial natural microbial community participates with the fungi to break down contaminants, eventually into carbon dioxide and water. Wood aromatic pollutants (toxic components of persistent pesticides; Battelle, 2000) capable of consuming Polyurethane in aerobic and anaerobic conditions such as found at the bottom of landfills. Mycofiltration is a similar process, using fungal mycelia to filter toxic waste and microorganisms from water in soil. Advantages advantages to bioremediation, which can be employed in areas that are inaccessible without excavation. For example, chlorinated solvents may contaminate acceptor or electron donor amendment, as appropriate, may significantly reduce contaminant concentrations after a long time allowing for acclimation. This is typically much less expensive than excavation followed by disposal elsewhere, situ treatment strategies, and reduces or eliminates the need for "pump and treat", a practice common at sites where hydrocarbons have contaminated clean Monitoring bioremediation: The process of bioremediation can be monitored indirectly by measuring the Oxidation/ Reduction potential or redox in soil and ground water together with pH, temperature, oxygen content, electron acceptor/donor concentrations, and concentration of breakdown products (e.g. carbon dioxide breakdown rate as function of the redox Process aerobic: Mycoremediation is a form of bioremediation in which fungi . The term mycoremediation refers specifically to the use of in bioremediation. One of the primary roles of fungi in decomposition, which is performed by the mycelium. The mycelium and acids that break down lignin and cellulose building blocks of plant fiber. These are organic compounds composed of long chains , structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutan strains have been reported to successfully degrade the nerve gases VX and conducted experiment, a plot of soil contaminated with diesel oil was inoculated with mycelia tional bioremediation techniques (bacteria) were used on control plots. After four weeks, more than 95% of many of the PAH (polycyclic aromatic hydrocarbons toxic components in the mycelial-inoculated plots. It natural microbial community participates with the fungi to break down contaminants, eventually into carbon dioxide and water. Wood-degrading fungi are particularly effective in brea aromatic pollutants (toxic components of petroleum), as well as chlorinated compounds (certain ; Battelle, 2000). Two species of the Ecuadorian fungus Pestalotiopsis are capable of consuming Polyurethane in aerobic and anaerobic conditions such as found at the is a similar process, using fungal mycelia to filter toxic waste from water in soil. Advantages - There are a number of cost/efficiency bioremediation, which can be employed in areas that are inaccessible . For example, hydrocarbon spills (specifically, petrolspills) or certain chlorinated solvents may contaminate groundwater, and introducing the appropriate electron r or electron donor amendment, as appropriate, may significantly reduce after a long time allowing for acclimation. This is typically much excavation followed by disposal elsewhere, incineration treatment strategies, and reduces or eliminates the need for "pump and treat", a practice ocarbons have contaminated clean groundwater. The process of bioremediation can be monitored indirectly by measuring the Oxidation/ Reduction potential or redox in soil and ground water together with content, electron acceptor/donor concentrations, and concentration of carbon dioxide). This table shows the (decreasing) b breakdown rate as function of the redox potential. Reaction Redox potential (E $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ 600 ~ 400 fungi are used to refers specifically to the use of , which is performed by the mycelium. The mycelium cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains , structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant. Certain and sarin. In one oil was inoculated with mycelia tional bioremediation techniques (bacteria) were used on control polycyclic aromatic hydrocarbons) It appears that the natural microbial community participates with the fungi to break down contaminants, eventually degrading fungi are particularly effective in breaking down), as well as chlorinated compounds (certain . Two species of the Ecuadorian fungus Pestalotiopsis are capable of consuming Polyurethane in aerobic and anaerobic conditions such as found at the is a similar process, using fungal mycelia to filter toxic waste There are a number of cost/efficiency bioremediation, which can be employed in areas that are inaccessible spills) or certain , and introducing the appropriate electron r or electron donor amendment, as appropriate, may significantly reduce after a long time allowing for acclimation. This is typically much incineration or other ex treatment strategies, and reduces or eliminates the need for "pump and treat", a practice The process of bioremediation can be monitored indirectly by measuring the Oxidation/ Reduction potential or redox in soil and ground water together with content, electron acceptor/donor concentrations, and concentration of). This table shows the (decreasing) biological Redox potential (Eh in mV) KARPAGAM ACADEMY OF HIGHER EDUCATION CLASS: I M.Sc Microbiology COURSE CODE: 18MBP204 Prepared by Dr.R.Radhakrishnan, Assi. Prof.,

Department of anaerobic: denitrification 2NO manganese IV reduction MnO iron III reduction Fe(OH) sulfate reduction SO4 2 fermentation 2CH This, by itself and at a single site, gives little information about the process of necessary to sample enough points on and around the contaminated site to be able to determine contours of equal redox potential. Contouring i software, e.g. using Kriging interpolation. If all the measurements of redox potential show that electron acceptors have been used up, it is in effect an Chemical analysis is also required to determine when the levels of contaminants and their breakdown products have been reduced to below regulatory Soil contamination:Soil contamination or soil of xenobiotic (human-made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals, or improper disposal of most common chemicals involved are petroleum hydrocarbons (such as naphthalene and benzo (a) pyrene) other heavy metals. Contamination is correlated with the degree of of chemical usage. The concern over soil contamination stems primarily from health risks, from direct contact with the contaminated soil, vapors from the contaminants, and from secondary contamination of water supplies within and underlying the soil. Mapping of contaminated soil sites and the resulting cleanup are time consuming and expensive tasks, requiring extensive amounts of geology, hydrology, Environmental Contamination, as well as an appreciation of the history of industrial chemistry. In North America and Western Europe many of countries in these areas having a legal framework to identify and deal with

$2NO3^- + 10e^- + 12H^+ \rightarrow N2 + 6H2O$ 500 ~ 200 $MnO2 + 2e^- + 4H^+ \rightarrow Mn2^+ + 2H2O$ 400 ~ 200 $Fe(OH)3 + e^- + 3H^+ \rightarrow Fe2^+ + 3H2O$ 300 ~ 100 $2^- + 8e^- + 10\ H^+ \rightarrow H2S + 4H2O$ 0 ~ −150 $2CH2O \rightarrow CO2 + CH4$ −150 ~ −220

This, by itself and at a single site, gives little information about the process of enough points on and around the contaminated site to be able to of equal redox potential. Contouring is usually done using specialized interpolation. If all the measurements of redox potential show that s have been used up, it is in effect an indicator for total microbial activity. Chemical analysis is also required to determine when the levels of contaminants and their reduced to below regulatory limits. Soil contamination or soil pollution is caused by the presence made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals, or improper disposal of n chemicals involved are petroleum hydrocarbons, polynuclear aromatic hydrocarbons (such as naphthalene and benzo (a) pyrene) solvents, pesticides, Contamination is correlated with the degree of industrialization of chemical usage. The concern over soil contamination stems primarily from health risks, from direct contact with the contaminated soil, vapors from the contaminants, and from secondary es within and underlying the soil. Mapping of contaminated soil sites and the resulting cleanup are time consuming and expensive tasks, requiring extensive hydrology, chemistry, computer modeling skills, and , as well as an appreciation of the history of industrial chemistry. Western Europe that the extent of contaminated land is best known, with many of countries in these areas having a legal framework to identify and deal with This, by itself and at a single site, gives little information about the process of remediation. It is enough points on and around the contaminated site to be able to s usually done using specialized interpolation. If all the measurements of redox potential show that for total microbial activity. Chemical analysis is also required to determine when the levels of contaminants and their is caused by the presence made) chemicals or other alteration in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals, or improper disposal of waste. The polynuclear aromatic sticides, lead, and industrialization and intensity of chemical usage. The concern over soil contamination stems primarily from health risks, from direct contact with the contaminated soil, vapors from the contaminants, and from secondary es within and underlying the soil. Mapping of contaminated soil sites and the resulting cleanup are time consuming and expensive tasks, requiring extensive skills, and GIS in , as well as an appreciation of the history of industrial chemistry. that the extent of contaminated land is best known, with many of countries in these areas having a legal framework to identify and deal with this KARPAGAM ACADEMY OF HIGHER EDUCATION CLASS: I M.Sc Microbiology COURSE CODE: 18MBP204 Prepared by Dr.R.Radhakrishnan, Assi. Prof., Department of environmental problem. Developing countries tend to be less tightly regulated despite some of them having undergone significant industrialization.

BIOLEACHING

- Bioleaching is cheaper than chemical extraction, safer for the environment, and more efficient in extracting metals with low concentration in ores.
- It is performed by iron and sulfide oxidizing bacteria or acid producing fungus.

- Bacteria recycle the major leaching reagent, like ferric iron, and perform further oxidation steps while gaining energy from the electron transfer.
- **ore leaching**: The process of recovering metals from ores by using a number of different techniques.

Microbial ore leaching (bioleaching) is the process of extracting metals from ores with the use of microorganisms. This method is used to recover many different precious metals like copper, lead, zinc, gold, silver, and nickel. Microorganisms are used because they can:

- lower the production costs.
- cause less environmental pollution in comparison to the traditional leaching methods.
- very efficiently extract metals when their concentration in the ore is low.

The Leaching Process

Bacteria perform the key reaction of regenerating the major ore oxidizer which in most cases is ferric iron as well as further ore oxidation. The reaction is performed at the bacterial cell membrane. In the process, free electrons are generated and used for the reduction of oxygen to water which produces energy in the bacterial cell.

Ores, like pyrite (FeS_2), are first oxidized by ferric iron (Fe^{3+}) to thiosulfate ($S_2O_3^{2-}$) in the absence of bacteria.

In the first step, disulfide is spontaneously oxidized to thiosulfate by ferric iron (Fe^{3+}), which in turn is reduced to give ferrous iron (Fe^{2+}):

(1)
FeS2+6Fe3++3H2O⟶7Fe2++S2O2−3+6H+spontaneousFeS2+6Fe3++3H2O⟶7Fe2++S2O32−+6H+spontaneous

Bacteria are added in the second step and recover Fe^{3+} from ferrous iron (Fe^{2+}) which is then reused in the first step of leaching:

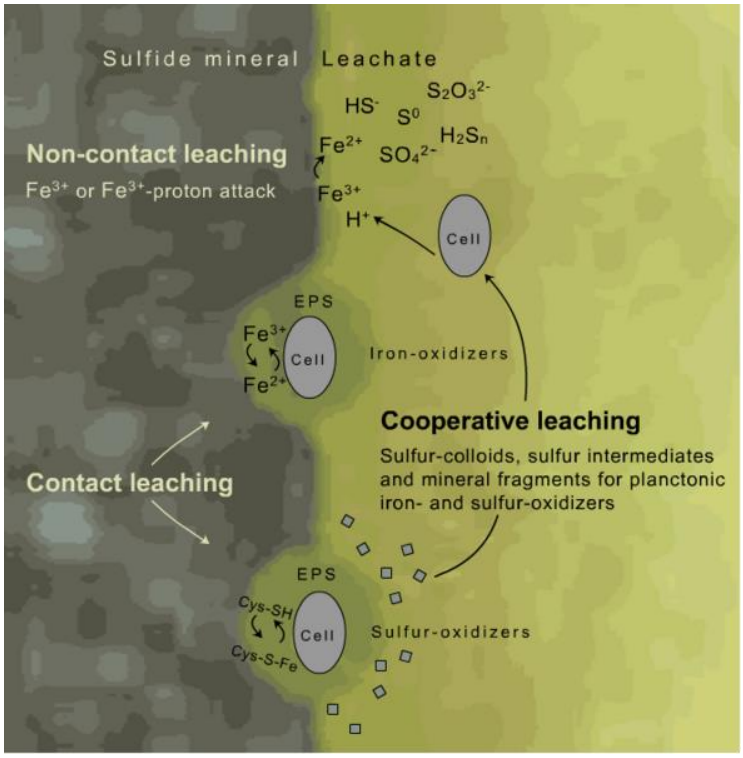


Figure: **Sulfide mineral bacterial leaching**: Bacterial cells oxidizing the ferrous iron back to ferric iron while using slightly different contact mechanisms with the metal.

(2) $4Fe2{+}{+}O2+4H{+}\longrightarrow 4Fe3{+}{+}2H2O$(iron oxidizers)$4Fe2{+}{+}O2+4H{+}\longrightarrow 4Fe3{+}{+}2H2O$(iron oxidizers)

Thiosulfate is also oxidized by bacteria to give sulfate:

(3) $S2O2{-}3+2O2+H2O\longrightarrow 2SO2{-}4+2H{+}$(sulfur oxidizers)$S2O32{-}{+}2O2+H2O\longrightarrow 2SO42{-}{+}2H{+}$(sulfur oxidizers)

The ferric iron produced in reaction (2) oxidized more sulfide as in reaction (1), closing the cycle and given the net reaction:

(4) $2FeS2+7O2+2H2O\longrightarrow 2Fe2{+}{+}4SO2{-}4+4H{+}2FeS2+7O2+2H2O\longrightarrow 2Fe2{+}{+}4SO42{-}{+}4H{+}$

The net products of the reaction are soluble ferrous sulfate and sulfuric acid.

The microbial oxidation process occurs at the cell membrane of the bacteria. The electrons pass into the cells and are used in biochemical processes to produce energy for the bacteria while reducing oxygen to water. The critical reaction is the oxidation of sulfide by ferric iron. The main role of the bacterial step is the regeneration of this reactant.

Copper leaching has a very similar mechanism.

Microorganisms Capable of Ore Leaching

Bioleaching reactions industrially are performed by many bacterial species that can oxidize ferrous iron and sulfur. An example of such species is *Acidithiobacillus ferroxidans*. Some fungi species (*Aspergillus niger* and *Penicillium*

simplicissimum) have also been shown to have the ability to dissolute heavy metals. When fungi are used, the leaching mechanism is different. The fungi use the acids that they produce in their metabolic reactions to dissolve the metal.

In general, bioleaching is cleaner and safer for the environment than chemical processing. However environmental pollution with toxic products, like sulfuric acid from the pyrite leaching, and heavy metals is still possible. Another drawback of microbial leaching is the slow rate at which microbes work.

OXIDATION OF MINERALS

Mineral Stability Are you glad to be finally done with igneous rocks? All those textures, all those compositions, all those minerals? Well the good news is that you are now done with igneous rocks. The bad news is that you still need to know Bowen's Reaction Series. In a very short period of time (2 lectures?) we will start talking about the next group of rocks (the sedimentary rocks). Before we get to them though, we have to discuss the origin of sediment. Sediment is a diverse group of materials that are initially unconsolidated (fragmented) and that can be converted to rock (sedimentary rock) under the right conditions. One of the ways that sediment is produced is through the break up (weathering) of other rocks. Since we left off with igneous rocks, we might as well use them as an example of how the weathering process works. Igneous rocks are composed of minerals that form from molten rock. Minerals that form at high temperature and/or high pressure do so because they are stable under those conditions. Olivine is very stable at 1800 °C, but at temperatures significantly less than that, like that at the surface of the Earth, olivine is unstable. Add water in the form of rain fall, and the mineral becomes very reactive. Olivine-rich rocks such as dunite, peridotite or basalt porphyry do not survive long at the surface of the Earth. Bowen's Reaction Series can also be considered a stability series. Those minerals that form first from a melt (e.g., olivine, pyroxene, Ca-plagioclase), are at the low stability end of the series while those that form last (e.g., quartz, muscovite), are at the high stability end of the series. Quartz is the most stable of the common minerals which explains why it is concentrated along Alabama's beaches, but as will be discussed shortly, even quartz can be GY 111 Lecture Notes D. Haywick (2008-09) 2 weathered under the right conditions. For the purposes of mineral stability, we will add four other minerals/mineraloids to our modified Bowen's Reaction Series. Kaolinite (a clay mineral) is more stable than muscovite. Limonite, hematite and bauxite are all more stable than quartz: B) Weathering There are three major types of weathering, although most textbooks only distinguish two. The first type is physical weathering and is defined as the mechanical breakup of rock. The second type of weathering is called chemical weathering. This is the most important process in soil formation (see the next lecture) and involves chemical changes during the breakup of rock. The last of the weathering types (not always distinguished in texts) is biological weathering. This involves the actions of plants and animals and is really just a combination of physical and chemical weathering. The main thing to remember about these types of weathering is that they all reduce rock into sediment. Physical weathering does this with little loss in volume. Chemical weathering may result in a significant loss in volume: GY 111 Lecture Notes D. Haywick (2008-09) 3 Physical weathering occurs everywhere, but is especially prevalent in areas of the Earth that are either very hot (e.g., deserts) or very cold (e.g., mountains, tundra). In hot areas, alternations between hot and cold conditions causes rock to expand and contract. It is felt by many geologists that this causes rocks to "sheet" off in a process called exfoliation (see the first of the two photos on this page). The second photo shows the end result of this process; spherical weathering results in rounded granite boulders atop mountains (see second photo on this page). By the way, both photos are from Doug's recent trip to Germany. Another type of physical weathering is called unloading. Granite forms well below the surface of the Earth in areas of fairly high pressure. When exposed at the Earth's surface, the rocks no longer feel the confining pressure and may tend to shatter because of the reduced pressure load. Unloading is really a problem in new mine shafts. Some granites (other rocks too, but granite is about the worst) will exploded in what is called a rock burst. This is just one of the hazards of being a miner. In cold climates, water is the major agent behind physical weathering. Liquid water expands when it freezes, so any water within cracks, fractures and joints (see an upcoming rock deformation lecture) exerts tremendous force when it freezes. Rocks can be literally split apart as the temperature drops. Mountains are particularly good areas to see the results of this frost heaving. The piles of rock that occur along the base of mountains (called scree or talus) was mostly derived from frost heave. GY 111 Lecture Notes D. Haywick (2008-09) 4 Physical weathering produces smaller bits of rock, but it doesn't actually change the composition of the rock. You would be able to recognize bits of granite or basalt or rhyolite. The most important thing it does is increase the relative surface area of the rock. The

surface area is the amount of contact area in an rock that is exposed to water. Water is the principle agent behind chemical weathering so the more surface area, the more contact area for chemical weathering. Or to put it more succinctly, the higher the surface area, the faster chemical weathering occurs. C) Chemical weathering reactions There are three major reactions responsible for chemical weathering: 1) solution (or dissolution) 2) oxidation 3) hydrolysis Solution occurs when a mineral dissolves. The next result is that you get ions in solution and nothing is left behind (example minerals: halite, calcite). Oxidation occurs when a mineral reacts with oxygen in the atmosphere or in water (example mineral: pyrite). Hydrolysis occurs when a mineral reacts with water (example minerals: orthoclase, pyrite, olivine). GY 111 Lecture Notes D. Haywick (2008-09) 5 We'll call it quits here for today. In the next lecture, we will discuss weathering a bit more as it applies to soil development. This will also include a discussion about the weathering of quartz. Under the right conditions, quartz undergoes chemical weathering (specifically solution).

Possible questions: Part- B (2 marks)

1. Write about microbial contamination in air.

2. Write about possible microbes in ore leaching.

3. What is leaching process.Define.

4. Define bioremediation.

5. Draw a neat sketch to sulfide mineral leaching.

Part –C (8 Marks)

1. Explain in detail about oxidation of minerals.
2. Discuss in detail about enumeration of bacteria in air.
3. Write in detail about bioleaching process.
4. Elaborate microbial indicator of air(microorganisms as biological indicators of air pollution.
5. Give a detailed account on air sanitation.

S.No	Unit II	Opt 1	Opt 2	Opt 3	Opt 4	Answers
1	_____ is a seasonal disease that can be deadly when becomes pandemic	Tuberculosis	Leprosy	Food poisoning	Influenza	Influenza
2	_____ air sampler is used in electrostatic precipitation of removing particles from air	Impinger	Orange	Sutton small volume	Litton large volume	Litton large volume
3	_____ is a severe respiratory disease caused by bacteria	Aspergillosis	Scarlet fever	Tuberculosis	Tetanus	Tuberculosis
4	_____ is an opportunistic fungal disease of human caused by inhalation of spores	Sporidiasis	Penicilliosis	Aspergillosis	Candidiasis	Aspergillosis
5	_____ is transmitted to humans by inhalation of faecal dust from pigeon droppings	Candidiasis	Histoplasmosis	Sporidiasis	Bacteraemia	Histoplasmosis
6	According to ambient air quality standards in India, amount of suspended particulate matters in a residential area is	200 µgm-3	400 µgm-3	600 µgm-3	800 µgm-3	200 µgm-3
7	Air doesn't have_____flora	Indigenous	Endogenous	Exogenous	Subgenous	Indigenous
8	Common source of air microflora is	Human	Soil	Industries	Vehicles	Both I & II
9	Cryptococcosis is caused by	*C.neoformans*	*C.licheniformis*	*C.pseudopodis*	*C.parvum*	*C.neoformans*
10	Efficiency of HEPA filter	75%	90%	95%	99.97%	99.97%
11	HEPA is an	Water filter	Soil filter	Air filter	Smoke arrester	Air filter
12	HEPA stands for	High Efficiency Particulate Air	High Energy Particulate Air	High Emission Particulate Air	High Efficient Polar Aerosol	High Efficiency Particulate Air
13	Human impact on the environment is termed as	Metagenic	Mutagenic	Carcinogenic	Anthropogenic	Anthropogenic
14	In India, amount of Carbon monoxide level permissible under ambient air quality standards for an industrial area is	5000 µgm-3	500 µgm-3	50 µgm-3	5 µgm-3	5000 µgm-3
15	Optimum rate of relative humidity for the survival of most microorganisms is between	10-20%	30-60%	40-80 %	80-100%	40-80 %
16	Percentage of oxygen is atmospheric air	40.9	60.9	75.9	20.9	20.9
17	Residue of solid material left after drying up of a droplet is known as	Mucus	Karyon	Oocyst	Droplet nuclei	Droplet nuclei
18	Size of a droplet nuclei	0.1-0.4 µm	1-4 µm	4-10 µm	10-40 µm	1-4 µm

19	Spores of_____travel over thousand kilometers	*Blastomyces*	*Fusarium*	*Puccinia graminis*	*Aspergillus*	*Puccinia graminis*
20	The commonest genera of fungi in indoor air are	*Saccharomyces*	*Penicillium*	*Rhizopus*	*Mucor*	*Penicillium*
21	The dominant microflora of outside air are	Fungi	Bacteria	Algae	Virus	Fungi
22	The layer nearest to the earth is called as	Troposphere	Ionosphere	Stratosphere	Thermosphere	Troposphere
23	Transmission of_____is mainly by inhaling the dust contaminated by animal products	Cholera	Anthrax	Typhoid	Dysentry	Anthrax
24	Valley fever or desert rheumatism is caused by	*Bordetella pertusis*	*Legionella pneuphila*	*Bacillus anthracis*	*Coccidioides immitis*	*Coccidioides immitis*
25	Vapours of_____are strongly germicidal	Poly ethylene glycol	Propylene glycol	Glycerol	Glycerin	Propylene glycol
26	Wavelength most effective in air sanitation by UV	254 nm	354 nm	454 nm	545 nm	254 nm
27	Which gas dominates composition of air?	CO2	Oxygen	Nitrogen	Hydrogen	Nitrogen
28	Which gas has greatest effect on global warming?	Ethane	Methane	CO2	Hydrogen	Methane
29	Which has greatest effect on ozone layer?	NO2	SO2	CFC	CO2	Chloro fluoro carbon
30	Which has the least presence in air?	Nitrogen	Oxygen	Argon	CO2	CO2
31	All are particulate pollutants except	dust	ozone	soot	Smoke	ozone
32	Fine organic and inorganic particles suspended in air is called _____	Particulate pollutant	gaseous pollutant	aerosol	none of these	aerosol
33	Which of the following is a secondary pollutant	CO2	CO	O3	SO2	O3
34	Carbonmonoxide is a pollutant because	it reacts with O2	It inhibits glycolysis	makes nervous system inactive	Reacts with haemoglobin	Reacts with haemoglobin
35	Air pollution is severe in _____	Cities	Agricultural field	Schools	Desert	Cities
36	The major pollutant from automobile exhaust is	NO2	CO	SO2	Soot	CO

37	A high biological oxygen demand	Water is pure	absence of microbial action	Low level of microbial pollution	High level of microbial pollution	High level of microbial pollution
38	Algal bloom results in _____	Global warming	Salination	Eutrophication	Biomagnification	Eutrophication
39	The green house gases, otherwise called radioactively active gases includes_____	CO2	O2	NO2	NO	CO2
40	According to EPA of USA , the following is not one of the six major pollutants?	Ozone	carbonmoxide	nitrogen oxides	carbondioxide	carbondioxide
41	Which of the following is an organic gas?	Hydrocarbons	Aldehydes	Ketones	Ammonia	Ammonia
42	Ozone is formed in the upper atmosphere by a photochemical reaction with _____	Ultraviolet solar radiation	Infra red radiation	Visible light	Gamma rays	Ultra violet solar radiation
43	Identify the term that describes an environment completely free of microorganisms	Antibiotic	Asepsis	Antisepsis	Probiotic	Asepsis
44	Human impact on the environment is termed as	Metagenic	Mutagenic	Carcinogenic	Anthropogenic	Anthropogenic
45	The major pollutant from automobile exhaust is	NO2	CO	SO2	Soot	CO
46	Size of a droplet nuclei	0.1-0.4 µm	1-4 µm	4-10 µm	10-40 µm	1-4 µm
47	Air doesn't have_____flora	Indigenous	Endogenous	Exogenous	Subgenous	Indigenous
48	The major pollutant from automobile exhaust is	NO2	CO	SO2	Soot	CO
49	The commonest genera of fungi in indoor air are	*Saccharomyces*	*Penicillium*	*Rhizopus*	*Mucor*	*Penicillium*
50	_____ is transmitted to humans by inhalation of faecal dust from pigeon droppings	Candidiasis	Histoplasmosis	Sporidiasis	Bacteraemia	Histoplasmosis
51	Ozone is formed in the upper atmosphere by a photochemical reaction with _____	Ultraviolet solar radiation	Infra red radiation	Visible light	Gamma rays	Ultra violet solar radiation
52	Which gas has greatest effect on global warming?	Ethane	Methane	CO2	Hydrogen	Methane
53	The major pollutant from automobile exhaust is	NO2	CO	SO2	Soot	CO
54	Optimum rate of relative humidity for the survival of most microorganisms is between	10-20%	30-60%	40-80 %	80-100%	40-80 %
55	Human impact on the environment is termed as	Metagenic	Mutagenic	Carcinogenic	Anthropogenic	Anthropogenic

56	Percentage of oxygen is atmospheric air	40.9	60.9	75.9	20.9	20.9
57	Which gas dominates composition of air?	CO2	Oxygen	Nitrogen	Hydrogen	Nitrogen
58	Air pollution is severe in _____	Cities	Agricultural field	Schools	Desert	Cities
59	Identify the term that describes an environment completely free of microorganisms	Antibiotic	Asepsis	Antisepsis	Probiotic	Asepsis
60	Fine organic and inorganic particles suspended in air is called _____	Particulate pollutant	gaseous pollutant	aerosol	none of these	aerosol

Importance of microbes in agriculture, Current agriculture problems. Bacterial diseases of agricultural crops -pathogens, symptoms and control measures with reference to paddy, cotton, maize, tomato, citrus, mango and potato. Plant protection –phenolics – phytoalexins and related compounds. Bioinsecticides –bacterial and fungal brief note.

Bacterial Disease # 1. Citrus Canker:

Fawcett and Jenkins (1933) reported that citrus canker disease is originated in India and Java because they have detected canker lesions on the oldest citrus herbaria kept at Royal Botanical Gardens in Kew. England (Citrus medica collected in India between 1827-1831, citrus aurantifolia collected in Java between 1842-1844).

Now the disease known to occurs almost in all citrus growing countries of the world. This disease affects cultivars and hybrids of citrus and citrus relatives including orange, grape-wine, pummeto, mandarin, lemon, lime, tangerine, sour orange and rough lemon. Because of its rapid spread, high potential damage and impact on export and domestic sales, the disease is a significant threat to all citrus growing countries.

Symptoms:

Plants infected with citrus canker disease have characteristic lesions on leaves, stems and fruits with raised brown, water soaked margins develop around the necrotic tissues (Fig. 3). A characteristic symptom of the disease on the leave is the yellow halo that surrounds lesion. These lesions start as pin point spots and attain a maximum size of 2-10 mm diameter.

Lesions became visible about 7-10 days after infection on the lower surface of leaves and soon appear on the upper surface. The lesion persists on twigs and branches for several years and support long term survival of the of the bacterium. Severely infected fruits can drop prematurely, leading to reduced yield. The internal quality of mature fruit with lesions is unaffected and is still edible and usable for juice.

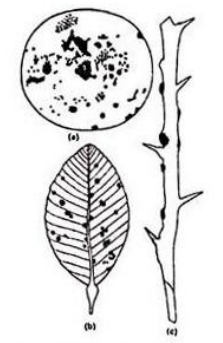


Fig. 3. Citrus canker. Lesions on (a) fruit (b) leaf and twig (c) ting.

Etiology:

(a) Causal organism Xanthomonas axonopodis pv. citri and Xanthomonas axonopoids pv. aurantiofolii.

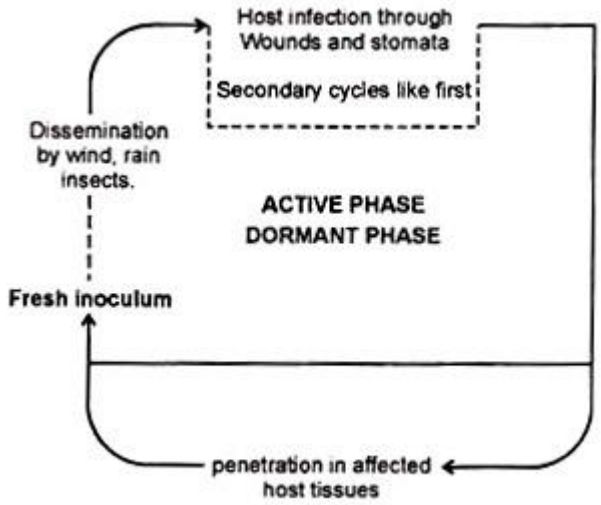
Xantotnonas is small, aerobic, rod shaped mobile bacterium. It is 1.5-2.0 x 0.5-.75 microns in size and has single polar flagellum. It forms chains and capsules but no spores.

Disease Cycle:

Infected twigs having old lesions are the main source of infection. These lesion ooze bacteria which are blown away by the wind or dispersed by rain. Healthy plants are infected by these bacteria which enters through stomata or wounds (caused by citrus leaf miner Phyllocnistis citrella in feeding activity).

The bacteria, on penetration into the host multiplies in the intercellular spaces, dissolves the middle lamella and gets established into the cortex. Lesion on healthy plants become visible about 7 to 10 days after infection on the underside of the leaves and soon thereafter on the upper surface.

Man is also an important agent of dissemination through infected nursery stock. High mean temperature (20-30°C) coincide with high rain fall favours the development of disease.



Disease cycle of Citrus canker.

Control Measures:

1. Eradication of infected trees and burning them.

2. Applying preventive sprays of copper based bacterisides e.g., Kocide 3000.

3. Pruning of infected twigs and leaves during the dry season and then spraying the trees with 1% Bordeaux mixture.

4. Spraying the antibiotics for e.g., streptomycin sulphate and phonomycin.

5. Strictly applying quarantine methods.

6. Replacing susceptible citrus cultivars with resistant cultivars.

7. Growing wind breaks to hinder inoculum dispersal.

8. Many areas of new infections of citrus canker are linked to human and mechanical transmission.

9. Developing transgenic plants for e.g., a Xanthomonas resistance gene from the rice has been transferred into a sweet orange.

Bacterial Disease # 2. Potato Scab:
It is a common bacterial disease of potato tubers. The disease occurs throughout the potato growing regions of the world. The pathogen affects beets, radish and other root crops.

It is a cosmetic disease with no or little effect on the yield. The major loss from the scab is lower market quality because tubers are unsightly and have poor customer appeal. Severe scab reduces the quality of the usable tubers, as more peeling is required.

Symptoms:
The first symptoms of the disease, are the appearance of small usually circular, brownish specks or spots (lesions) on the young tubers. These spots soon enlarge, darken, and become corky.

Certain spots may merge to form large scabby areas. These spots may be so numerous as to give a russeted appearance to entire tuber. Pitted scab develops where lesions develop up to half inch deep. These deep spots are dark brown to black in colour.

Etiology:
Causal organism: Streptomyces scabies.
It is a saprophytic bacterium. It is represented by branched mycelium with few or no cross walls. The mycelium produces spores on specialised, spiral sporogenous hyphae. The spores produce one or two germ tubes (Fig. 4).

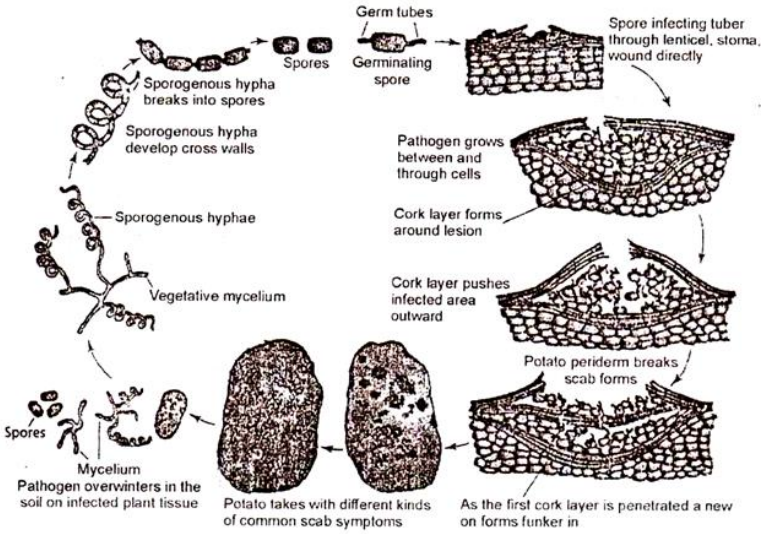


Fig. 4. Disease cycle of Potato scab caused by *Streptomyces scabies.*

Disease Cycle:

The disease spreads through soil water, by wind blown soil and infected seed tubers. The pathogen overwinters in the soil on infected plant tissues. The pathogen can also survive in the digestive tract of animals.

The bacterium produces spores which penetrate the tissues of young tuber directly, through lenticels, wounds, stomata and form a mycelium. After penetration the pathogen feeds on the peridermal layers causing death of the cells.

Living cells surrounding this tissue divide rapidly and produce several layers of cork cells that isolate the pathogen and other plant cells. These corky layers pushes the infected area outwards. As the potato periderm breaks, a scab is formed.

The vegetative mycelium produce spiral sporogenous hyphae which break into spores. These spores spread the disease. As the first cork layer is penetrated a new one forms below repeating the cycle and resulting in the development of large scab lesions. 5.2 to 8 soil pH favours the spreading of disease (Fig. 4).

Control Measures:

1. Use of certified scab free seed potatoes.

2. Keeping soil pH at or below 5.2 will suppress scab.

3. Avoid light textured soil.

4. Planting resistant cultivars, Variety Nooksack is highly resistant to disease.

5. Crop rotation for e.g., rotation of potato crop with alfalfa reduces the scab severity.

6. Keeping soil moist during early tuber development.

7. Chemical pentachloronitrobenzene (PCNB) also known as Blocker (Amvac) or Maneb-zinc dust can be used for seed treatment.

8. Biological control of scab with streptomyces phage.

Bacterial Diseases Of Tomato: Bacterial Spot, Bacterial Speck, Bacterial Canker

Introduction

Three bacterial diseases are common in Ontario tomato fields: bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria;* bacterial speck, caused by *Pseudomonas syringae* pv. *tomato*; and bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis*.

In Ontario, bacterial disease is present at some level every season, though not always at destructive levels. However, when conditions are optimal for bacterial disease, losses in marketable yield can be up to 60% in some fields.

Damage from these diseases may range from a light spotting of the foliage to almost complete defoliation of the plant, with corresponding impacts on photosynthesis and production potential. When present, fruit lesions disfigure and reduce the marketability of both fresh-market and processing fruit (especially in whole-pack or diced product) and interfere with peeling. Defoliation exposes the fruit, resulting in sunscald and poor colour. Secondary rots can also develop.

Both fresh-market and processing growers may incur higher sorting costs due to fruit lesions. Processing growers also face the risk of increased tare penalties and the possibility of not meeting their contracted tonnage. Depending on the product being produced, bacterial disease may result in lower solids, increased costs, slower factory operations and reduced peeled recovery for the processors. Processors also face the risk of falling short of their packing goals.

Management of tomato bacterial diseases must focus on prevention and must start well before transplanting. Seed suppliers, transplant growers, field growers, processors, researchers, extension specialists and crop advisors all have a part to play.

The Pathogens

Bacterial pathogens need moisture to multiply. Wet conditions in the plant canopy due to rain, fog, dew, high humidity or irrigation give the bacteria a suitable environment for growth. Each pathogen has a particular temperature range, in which it is at its peak rate of growth and infection (see Table 1, below). The pathogens multiply much more slowly outside this optimum range.

Disease	Causal organism	Optimum temperature
bacterial spot	*Xanthomonas campestris* pv. *vesicatoria*	24°C- 30°C
bacterial speck	*Pseudomonas syringae* pv. *tomato*	18°C- 24°C
bacterial canker	*Clavibacter michiganensis* subsp. *michiganensis*	24°C- 32°C

Table 1. Optimum growth temperature ranges for bacterial pathogens of tomato

Bacterial Spot

Symptoms

The bacterial spot pathogen may produce lesions on all aboveground parts of the plant - leaves, stems, flowers and fruit. It is difficult to reliably distinguish bacterial spot from bacterial speck based on visual symptoms, especially in the early stages.

Initial leaf symptoms are small, circular-to-irregular, dark lesions, which may be surrounded by a yellow halo. The lesions tend to concentrate on the leaf edges and tip and may increase in size to a diameter of 3-5 mm. Infected leaves may develop a scorched appearance. When spots are numerous, foliage turns yellow and eventually dies, leading to defoliation of the lower portion of the plant.

Figure 1: Bacterial spot lesions on tomato leaves.

Figure 2: Tomato plant showing defoliation and fruit symptoms due to bacterial spot.

Lesions on pedicels may cause flower abortion, resulting in lost yield and split sets of fruit.

Fruit lesions are initiated only on green fruit, most likely because infection occurs through fruit hairs, which are present only on immature fruit. On fruit, the first symptoms are small, dark brown-to-black, raised spots. The lesions also may have a white halo, similar to the bird's-eye spotting seen with bacterial canker. As the fruit ages, the white halos disappear. In contrast, bacterial canker fruit lesions retain their white halo. Bacterial spot lesions may increase in size to 4-6 mm in diameter and become brown, greasy-looking and sometimes scabby.

Inoculum and Spread

The major sources of infection for these bacteria are thought to be seed and infected crop debris. Like the bacterial speck pathogen, they also may be present on volunteer tomato plants and on the surfaces of contaminated equipment (farm machinery, racks, greenhouse structures, tools). The bacteria are spread primarily by splashing water and wind-driven rain or mists produced during storms. In the field, spread by equipment or workers is probably of lesser importance than it is in the greenhouse, unless wounds are being opened up at the same time, as in a pruning operation or when plants are injured by a cultivator..

Bacteria enter the plant through natural openings (stomates and hydathodes) or wounds caused by wind-driven soil, insects or mechanical damage (handling, wind whipping, high pressure sprayers).

Causal Organism

The bacteria that cause bacterial spot are called xanthomonads. Recent taxonomic studies have indicated that these bacteria belong to one of four groups: A, B, C and D. The original (and still valid), taxonomic name given to these bacteria was *Xanthomonas campestris* pv. *vesicatoria*, but recent work has indicated that each group may represent a separate species. Group D has become the predominant form in Ontario. This is of concern, as recent research has shown it to be a particularly aggressive form, which can overwinter under southern Ontario conditions. The bacterial spot-causing xanthomonads also have been

classified into races, some of which infect only tomato or pepper, and some of which can infect both crops.

The genetic variability of the bacterial spot-causing xanthomonads makes it difficult for plant breeders to develop stable resistance in tomato varieties and for pathologists to develop control measures. If genetic resistance or chemical controls are only effective on one strain, the pathogen population will simply shift to the more tolerant strains.

Bacterial Speck

Symptoms

Bacterial speck lesions may occur anywhere on the foliage, stems or fruit. Symptoms are very difficult to visually distinguish from bacterial spot and can be confused with young, early blight lesions. On leaves, symptoms appear as black specks, usually no more than 2 mm in diameter, which are usually surrounded by a yellow halo. Speck lesions sometimes cause distortion of the leaf, as the infection restricts the expansion of leaf tissue. Lesions are often concentrated near leaf edges, and in some cases, leaf margin burn resembling bacterial canker may occur. When numerous, lesions may coalesce, and entire leaflets may die. Severely infected seedlings may become stunted.

Only green fruit less than 3 cm in diameter is susceptible to infection by the bacterial speck pathogen. Small (less than 1-3 mm), slightly raised black specks develop and are often surrounded by a narrow green to yellow halo. Lesions are usually superficial and can be scraped off with a fingernail. Red fruit are not susceptible to infection, likely due to a lack of entry points for bacteria; fruit hairs, which may break and allow bacteria to enter, are only present on young fruit. On fruit previously infected, black lesions remain after ripening.

Inoculum and Spread

The sources of bacterial speck inoculum and the methods of spread are the same as those for bacterial spot (see page 2). Studies have shown that the speck organism can survive in the crevices and cavities of the tomato seed coat for up to 20 years.

Causal Organism

Bacterial speck is caused by *Pseudomonas syringae* pv. *tomato*. Two races are present in Ontario, race 0 and race 1. The Pto gene, discovered by Ontario researchers, confers resistance to race 0.

This bacterium produces a number of compounds that help it infect and obtain nutrients from the tomato plant. One of these compounds is the plant-specific toxin coronatine, which is responsible for the yellow halo surrounding leaf lesions and the stunting of young seedlings. The majority of bacterial speck strains that have been isolated from Ontario tomato fields are either resistant to or tolerant of copper-based bactericides.

Bacterial Canker

Symptoms

Bacterial canker, which may occur in tomato as a primary (systemic) or secondary (foliar) infection, shows a wide range of symptoms.

Primary infections originate from infected seed or from invasion of the vascular tissue of young seedlings. Symptoms, which may not show up until several weeks after infection, initially appear as wilting and downward turning of the lower leaves. The wilting generally progresses upwards, unless the site of infection is in the upper part of the plant. Wilting is often seen only on one side of the leaf or one side of the plant. Plants may collapse and die, especially if infected at a very early stage. Generally, plants survive but are stunted, showing some or all of the symptoms described here, depending on their environment and stage of growth.

Tomato foliage infected with the canker organism has distinctive black leaf edges with no spotting on the interior of the leaves. Sometimes a thin yellow border is present between the dead leaf margins and healthy tissue.

If an infected stem is cut lengthwise, a light brown discolouration may be present in the vascular tissue, most noticeable at nodes and just above the soil line. As the disease progresses, this turns reddish-brown. Light coloured streaks are often visible on the outside of the stem. These may later darken and break open into cankers. With severe infections, a yellow ooze may exude from a cut stem when it is squeezed.

Fruit may develop relatively small spots with light brown centres, generally surrounded by a greasy white halo (3-6 mm in diameter). These are known as bird's-eye spots. With bacterial canker lesions, this white halo generally remains as the fruit ripens, while in the case of bacterial spot, it disappears with time. Bacterial canker may also cause a darkening of the vascular tissues within the fruit. The fruit may show a black peppering at the vascular bundles under the calyx scar. Canker bacteria can grow in the vascular bundles within the fruit, all the way to the seed. This can result in visible yellowish strands from the stem to the seeds and internal infections in the seed.

With a secondary foliar infection, leaves develop brown-black margins with a thin, yellow (chlorotic) band. Leaflet edges may curl upwards. Fruit may show bird's-eye spotting, as in a systemic infection. Secondary infections (no vascular system involvement) often have minimal impact on the crop, especially when initiated later in the season.

Inoculum and Spread

Infected seed is probably the major source for primary (systemic) infections. The bacteria can be present on the surface of the seed as well as within the innermost layer of the seed coat. This makes the canker organism harder to eradicate with seed treatments than the spot and speck pathogens.

The organism can also be introduced from infected crop debris, weed hosts or volunteer tomatoes, and contaminated equipment. Studies in the U.S. northern Midwest have shown that it can overwinter on infected debris. However, crop rotation and tillage before planting should reduce the risk of infection from this source.

The canker bacteria enter the plant through natural openings and wounds, including root wounds. Pruning or transplant clipping operations can introduce the bacteria directly into the vascular system, resulting in the more serious systemic infections.

Infections spread through splashing water, wind-driven rain and the fine water droplets or aerosols produced during storms. In the field, bacteria transfer by machinery or workers is probably not as significant as in the transplant greenhouse where plant density is high and growth conditions for the bacterium are optimal.

Causal Organism

Bacterial canker is caused by *Clavibacter michiganensis* subsp. *michiganensis*.

Diseases and Disorders With Similar Symptoms

Early blight and septoria leaf spot are two common fungal diseases that cause spots on tomato foliage. Young early blight lesions can resemble bacterial lesions and often have a yellow halo. Look for the dark concentric rings that indicate early blight. Early blight lesions enlarge and become angular over time. Septoria lesions can be distinguished due to their light tan centres containing tiny black dots (pycnidia). These can be seen clearly with a hand lens. Yellowing of the foliage is rarely present with septoria leaf spot until lesions become numerous. Neither fungal disease produces the small black fruit lesions typical of speck or spot, or the bird's-eye spotting of canker.

Ozone injury may also cause spotting on tomato foliage, along with yellowing, purpling, glazing or curling of leaves. Recently matured leaves are most susceptible, and injury may also be present on nearby sensitive species. On fruit, hail damage and insect stings can resemble bacterial lesions.

The wilting symptoms of a systemic bacterial canker infection may be confused with verticillium wilt, which also may cause wilting on one side of the plant or leaf and browning of the vascular system near the soil line. Verticillium wilt typically causes significant yellowing of the foliage and V-shaped lesions extending out to the leaf tips. Another wilt disease, bacterial wilt, causes a more extensive discolouration of the vascular and stem tissue, which may extend well below the soil line. Bacterial wilt does not overwinter in Ontario, and so would only be found on transplants from southern US growing regions. (One race of bacterial wilt (race 3 biovar 2) may be able to overwinter in northern regions, but this form has not become established in the US or Canada. This race is a regulated and quarantined pest.) Lightning injury and walnut wilt (of plants growing near black walnut trees) are other common causes of wilt symptoms.

Diagnosis

Sampling

For plant disease diagnosis, choose representative plants showing early symptoms. Submit as much of the plant as is practical, or several plants showing a range of symptoms.

Wrap plants in newspaper and put in a plastic bag. Tie the root system off in a separate plastic bag to avoid drying out and contamination of the leaves by soil. Do not add moisture, as this encourages decay in transit. Cushion specimens and pack in a sturdy box to avoid damage during shipping. Protect specimens from excessive heat or freezing and deliver to the diagnostic lab as soon as possible by first class mail or courier at the beginning of the week.

Hot Water Treatment

Place the seed in a loosely woven cotton bag (such as cheesecloth). Leave lots of room in the bag for the seed to move around. Prewarm the seed for 10 min in 37°C (100°F) water. Place pre-warmed seed in hot water at 50°C (122°F) for 25 min, monitoring the temperature

constantly. Cool immediately by placing seed in cold water for 5 min. Dry thoroughly. Expect to lose 5%-10% of viable seed.

Transplant Production

By following an effective disinfection protocol, the seed supplier has done everything possible to ensure clean seed. Now the transplant grower must take steps to prevent the seed or seedlings from becoming infected in the greenhouse environment.

Transplant growers must maintain good sanitation practices. There is a wide range in types of transplant production facilities across the fresh-market and processing tomato industries, but in general, sanitation practices must include the following steps:

- Remove all plant material from the greenhouse before starting a new crop.
- Control weeds in and around the greenhouse.
- Start with sterile potting mix and trays.
- Use new, sterile trays if feasible, but if reusing trays, sanitize effectively by solarization or washing with disinfectant (for more information, see the *Transplant* section of OMAFRA Publication 363, *Vegetable Production Recommendations*).
- Disinfect racks, tools, equipment and greenhouse surfaces before the growing season - wooden racks must be soaked in the disinfecting solution for a minimum of 1 hr.
- Avoid contact between seed lots - sanitize equipment and hands between lots; physically separate seed lots in the greenhouse.
- Avoid contact between tomato and pepper seed and plants - sanitize equipment and hands after handling, physically separate crops in the greenhouse (ideally grow in separate facilities).
- Minimize handling and human traffic in the greenhouse.

An important cultural practice for disease control during transplant production is minimizing leaf wetness. Attempt to reduce the number of hours leaves are wet through timing of watering, control of relative humidity, ventilation and heating.

Use low pressures when watering to minimize plant damage and splashing of water droplets that can contain bacterial cells.

Do not handle wet plants, and ensure foliage is dry for shipping. Wet foliage and dripping water in plant trailers is a very effective way to spread disease and promote bacterial growth.

Field Production

Since all three bacterial pathogens can survive in crop debris, rotate tomatoes with non-host crops. If tomato crop debris is well worked into the top 15 cm (6 in.) of soil to speed decomposition, a 3-year rotation should be sufficient. Control weeds and volunteer tomatoes in and around the field, as they can act as reservoirs of disease.

Ensure good drainage and adequate fertility. After heavy rains, get excess water off the field as soon as possible, and address problem areas with drop pipes or other measures.

Separate plant lots into different fields if possible. Consider using tall, barrier crops between plant lots and neighbouring fields, but take care to maintain good air circulation within the field.

Try to avoid wet foliage during transplanting. It is difficult to dip the plug trays to wet the plugs without wetting the foliage, but it is beneficial if it can be achieved.

Ideally growers would be able to keep workers out of the field when the foliage is wet. This, however, is not always practical. Keep in mind that earlier infections have more time to cause damage and that fruit lesions are initiated on young, green fruit. Late-season foliar symptoms are not a major concern.

Low-pressure systems are better if overhead irrigation is used, as they minimize splashing and plant damage. The potential for overhead watering to spread disease must be balanced with the potential benefits from irrigation if an overhead system is all that is available. Plants under stress are less able to withstand a disease outbreak.

Genetic

Bacterial spot

There have been breeding programs for bacterial spot for at least 20 years. The work is complicated by the fact that there are at least 4 groups (possibly separate species) of bacterial spot affecting tomato. Recently, tomato lines have been identified that have resistance to multiple races of bacterial spot. With the diversity of the spot pathogen, it remains to be seen whether stable field resistance will be achieved in commercial cultivars.

Bacterial speck

The Pto gene, which confers resistance to bacterial speck race 0, is present in many commercial cultivars. To date, there has not been any confirmed breakdown of resistance to this gene. Race 1 of bacterial speck, which can infect plants with the Pto gene, has not been an important problem commercially.

Bacterial canker

Tomato cultivars with some resistance or tolerance to canker have been introduced, but there is little significant tolerance in commercial tomato varieties. Recent research has identified 2 genes for resistance to bacterial canker. Further work is needed before cultivars with greatly improved resistance are available to tomato growers.

Chemical

Fixed copper bactericides are currently the only effective registered control products for bacterial disease on tomatoes. However, bacterial speck populations in Ontario have shown widespread resistance to copper, and in some areas of the US, bacterial spot has also developed resistance. Despite this, copper is still a useful tool for managing bacterial spot and canker in Ontario.

Research at Ridgetown College (University of Guelph), Agriculture and Agri-Food Canada and elsewhere has shown that foliar-applied fixed copper sprays will reduce the number of bacterial cells on tomato foliage. Depending on the amount of bacteria present and the size and rate of growth of the plants, spray intervals of 7 days or less may be required. Copper sprays are less effective, to the point of ineffectiveness, when spray intervals are extended. Good spray coverage and the use of recommended rates are also very important.

The effect of the pH of the spray solution on the effectiveness of fixed copper formulations for bacterial disease control is unclear. The activity of fixed copper on the bacteria is due to free copper ions in the spray solution, the concentration of which changes with pH. Commercial pesticides, however, are generally formulated with buffers, surfactants and other additives to ensure pesticide efficacy under the normal range of application conditions (including spray solution pH). Follow manufacturer's recommendations for mixing and application, as high concentrations of copper ions can damage plant tissue.

Bacteria reproduce very quickly. Although foliar sprays may clean the surface bacteria from a leaf, within a short period of time the bacteria inside the leaf and those not controlled on the foliage (due to incomplete spray coverage) can build up population levels that can cause an outbreak.

In the past, copper spray programs may not have been applied at the right time to be fully effective. Bacterial disease does not affect each grower every year. It has been a common practice to begin an intensive copper spray program once lesions are present. We now know that starting a control program after symptoms have appeared is too late. It takes an incredibly high density of bacterial cells on the plant before symptoms are visible, and efforts to eradicate bacteria when they are at such high population levels are destined to fail.

The most current recommendations for copper spray programs for greenhouse transplant production and field production are found in OMAFRA Publication 363, *Vegetable Production Recommendations*. These recommendations outline a preventative program and must be followed closely to be effective. Making the decision to initiate a bacterial disease spray program when no symptoms are present can be difficult. However, with clean seed, the use of the non-chemical management practices described above, and preventative copper applications in the transplant greenhouse and the field, relatively few sprays are required.

Many research trials across North America have shown that tank-mixing mancozeb with copper enhances bacterial disease control.

See OMAFRA Publication 363, *Vegetable Production Recommendations*, for more information on registered products and for updates to bacterial disease control recommendations. Consult your processor or buyer, if applicable, as they may have specific restrictions or recommendations regarding pesticide use.

The Future

Some of the technologies being explored are outlined below:

- **Bacteriophages.** Phages are viruses that infect bacteria. Scientists are able to produce mixtures of phages that are specific to bacterial populations present in a given growing area. One of the major challenges with this technology is to maintain a viable phage population on the crop foliage for an adequate length of time.
- **SAR products.** SAR, or systemic acquired resistance, products actually trigger or enhance the plant's natural defences against infection. Some of these products have shown promise in reducing bacterial disease on tomatoes and are now in use in some tomato-growing areas.
- **Bacteriocins.** These are substances produced by plant pathogenic bacteria that antagonize other closely related bacteria. Avirulent (non-disease producing) variants of the bacterial pathogens that produce the desired bacteriocins would have to be developed, along with a way to formulate them for crop use.
- **Microbial biocontrol agents.** Research is also under way to develop biological control agents, some of which are commercially available in the US for other crops and other diseases, for use on Ontario's tomato crop.

Diseases of Cotton:

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1. Angular Leaf Spot or Black Arm Disease
2. Vascular Wilt Disease
3. Grey Mildew or Dahiya Disease
4. Anthracnose Disease
5. Root Rot Disease
6. Boll Rot Disease
7. Leaf Spot or Blight Disease

8. Reddening or Lalya Disease
9. 2, 4-D Injury Disease
10. Tobaaco Streak Virus Disease

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1. Angular Leaf Spot or Black Arm Disease:

Causal Organism:

Xanthomonas axonopodis pv. malvacearum (Smith) Vauterin.

ADVERTISEMENTS:

Symptoms:

Small water-soaked spots appear on the under surface of cotyledons, which may dry and wither. Such spots also appear on the leaves. They become angular bound by veinlets and turn brown to black in colour. Several small spots may coalesce. The infected petiole may collapse. Elongated, sunken and dark brown to black lesions appear on stem, petioles and branches.

The young stems may be girdled and killed in the black arm phase. Sunken black lesions may be seen on the bolls. Young boll may fall-off. The attacked stem becomes weak. Bacterial slime is exuded on the brown lesions. Discolouration of lint may take place.

Angular leaf spot symptoms

Etiology:

ADVERTISEMENTS:

The bacterium is rod-shaped. It occurs singly or in pairs, is capsulated but forms no spores, and is motile by one polar flagellum. Stain reaction is gram negative. The bacterium is aerobic.

Perpetuation:

The pathogen can remain as slimy mass inside the seed or on the fuzz. The disease may be carried over through infected leaves, bolls and twigs on the soil surface. The secondary infection is through water, wind.

Management:

1. Field sanitation.

2. (a) Externally seed borne infection can be eradicated by delinting the seed with Cone H_2SO_4 for 5 minutes, wash with lime solution to neutralise the effect and finally washing with running water to remove the residue and drying seeds.
(b) Internally seed borne infection can be eradicated by soaking seeds overnight in 100 ppm streptomycin sulphate or Agrimycin.

3. Secondary spread of the disease can be controlled by spraying the crop with streptomycin sulphate 100 ppm + Copper oxychloride (0.25%) at an interval of 15 days.

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2. Vascular Wilt Disease:

ADVERTISEMENTS:

Causal Organism:

Fusarium oxysporum f. sp. vasinfectum (Atk.) Snyder and Hansen.

Symptoms:

Wilt is restricted to black cotton soils with pH 7.6-8.00. It is rare in light to loam soils. The disease appears at all the stages of plant growth. In seedling stage, there is yellowing of cotyledons, browning of petioles, followed by death and falling of affected leaves. In young and adult plants, there is loss of turgidity, drooping of leaves and tender shoots, yellowing, browning and finally death of the plants.

ADVERTISEMENTS:

Etiology:

Mycelium is septate, hyaline and intravascular. The fungus is facultative parasite and produces three types of spores, Micro-conidia, which are one or bicelled, oblong, hyaline and borne on short conidiophores, Macro-conidia are 3 to 6 celled, chlamydospores, which are hyaline, spherical and thick walled.

Perpetuation:
Primary infection takes place through soil borne inoculums.

Management:
1. Field sanitation, crop rotation and mix cropping are useful for reducing the incidence.

2. Use of resistant varieties. G. arboreum and G. herbaceum are susceptible whereas G. hirsutum and G. barbedanse are immune.

3. American varieties are resistant to wilt in India.

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3. Grey Mildew or Dahiya Disease:

Causal Organism:
Imperfect stage. Ramularia areola Atk.

Perfect stage. Mycosphaerella areola.

Symptoms:
The fungus usually attacks the older leaves causing irregular to angular, pale, translucent spots. They are usually restricted by the vein lets and appear mostly on the lower surface of the leaf though occasionally on the upper surface. A few to over a hundred spots may be found on a single leaf. In severe infections the leaves turn yellowish brown and fall off prematurely.

Grey mildew symptom

Etiology:

A frosty or whitish grey mildew growth consists of conidia and conidiophores and mycelium of the fungus. The mycelium is endophytic, septate. The conidiophores are short, septate, branched at the base. The conidia are borne singly or in short chains at the tip of the conidiophores and are colourless, irregularly oblong, with pointed, rounded, or flattened end, unicellular to three septate.

Management:

1. Destruction of infected plant debris.

2. Dust the crop with 300 mesh sulphur at the rate 20 kg/ha or spray crop with 0.05% propiconazole.

1. Bacterial Stalk Rot: Common Maize Diseases

Causal organism: Erwinia carotovora, Erwinia chrysanthemi

Symptoms

- The basal internodes develop soft rot and give a water soaked appearance. A mild sweet fermenting odour accompanies such rotting.
- Leaves some time show signs of wilting or water loss and affected plants within a few days of infection lodge or topple down.
- Ears and shank may also show rot. They fail to develop further and the ears hang down simply from the plant

Control measures

- Use of disease resistance varieties, i.e. Hybrids Ganga Safed-2, DHM 103, show significantly less disease incidence than other hybrids.
- Avoid waterlogging and poor drainage.

READ MORE: Top 10 Concerns Of Young Farmer

2. Black Bundle Disease and Late Wilt: Common Maize Diseases

Causal organism: Cephalosporium maydis, Caphalosporium acremonium

Symptoms

- The disease kills the plant prematurely after flowering. Infected plants do not show symptoms until they reach to tasseling.
- Wilting generally starts from the top leaves, Leaves become dull green, eventually loose colour and become dry.
- In advance stages the stalk loses its healthy green colour, lower portions become dry,shrunken with or without wrinklings, hardens and turn purple to dark brown which in more prominent on lower internodes.
- When split open diseased stalks, show brown vascular bundles starting in the underground portion of the roots.
- Diseased plants produce only ears with undeveloped shrunken kernels.
- In severe cases affected plants remain abortive causing 100 per cent loss.
- Cephalosporium maydis is primarily soil borne and may infect young maize plants more readily than other plants through roots or mesocotyl. In case of C. acremonium only vascular burdles get blackened.

Control measures

i. Use of resistant varieties like Ganga Safed 2.
ii. Crop sanitation, crop rotations.
iii. Avoiding water stress at flowering.
iv. Seed treatment with Thiram or Captan 3g/kg seed.

3. Charcoal-Rot: Common Maize Diseases

Causal organism: Macrophamina phaseolina

Symptoms

- The characteristic symptoms of the disease become apparent as the plants approach maturity. The disease generally appears early after flowering.
- Plants affected by M. phaseolina show evidence of pre-mature ripening. The out sides of lower internodes become straw coloured. The pith becomes badly disintegrated.
- The infected stalks may split longitudinally into a mass of fibres.
- A distinguishing character of the disease is the presence of the small black sclerotia in the pith of the affected stalks. Roots are also invaded and show black sclerotia in the disorganised tissue.

Control measures

i. Regular irrigations particularly during flowering time should be provided.
ii. Use resistant varieties like DHM 103, Ganga Safed – 2 and avoid sowing of suceptable varieties like DHM 105.
iii. Seed treatment with Carbendazim or Thiram 3g/kg seed is effective.
iv. Field sanitation, crop rotation should be followed.

4. Common Rust: Common Maize Diseases

Causal organism: Puccinia sorghi

Symptoms:

- Circular to elongate golden brown or cinnamon brown, powdery, erumpent pustules appear on both leaf surfaces
- As the crop matures brownish black pustules containing dark thick walled two celled teliospores develop. In severe cases infection spreads to sheaths and other plant parts.

Control measures

i. Plant hybrids like Deccan, Ganga-5, Deccan Hybrid Makka-103 and DHM – 1 which are resistant to this disease to minimise the disease intensity.
ii. Spray Mancozeb 2.5g/lit or Dithane M-45 spray can be taken (0.4%) as soon as first symptoms are observed and it can be repeated at 10 days interval till flowering.

5. Downy Mildews: Common Maize Diseases

a) Sorghum downy mildew:

Causal organism: Perenosclerospora sorghi.

Symptoms

- Early maize crops escape infection because by the time conidia are produced they develop resistance on the collateral host susceptibility occurs only upto about 15 days of age.
- In susceptible seedling plants, less than 4 weeks after seedling infection becomes systemic in all growth, subsequent to downward growth of mycelium and colonization of shoot apex (growing point). All Peronosclerospora spp. induce both local and systemic infection.
- Malformation of tassels in infected plants.
- Chlorosis, white stripes, stunting with downy fungal growth on both leaf surfaces are the characteristic symptoms.

b) Brown stripe Downy mildew:

Causal organism: Sclerophthora rayssiae.

Symptoms

i. Disease symptoms have been observed only on leaves. They are vein limited.
ii. Wilting generally starts from the top leaves; Leaves become dull green, eventually loose colour and become dry.
iii. Chlorotic stripes, 3-7 mm wide will develop and they further extend in parallel fashion and may in severe cases cover the entire leaf lamina.
iv. Severe infection also incites blotching. The stripes in advanced stage become necrotic with
v. purple or reddish colour and present a burnt appearance.

Bacterial canker

Disease symptoms

- The disease is noticed on leaves, leaf stalks, stems, twigs, branches and fruits, initially producing water soaked lesions, later turning into typical canker.
- On leaves, water soaked irregular satellite to angular raised lesions measuring 1-4 mm in diameter are formed. These lesions are light yellow in colour, initially with yellow halo but with age enlarge or coalesce to form irregular necrotic cankerous patches with dark brown colour.
- On fruits, water-soaked, dark brown to black coloured lesions are observed which gradually developed into cankerous, raised or flat spots. These spots grow bigger usually up to 1 to 5 mm in diameter, which covers / almost the whole fruit.

- These spots often, burst extruding gummy substances containing highly contagious bacterial cells.

Survival and spread

- In lesions on plant parts and can also survive for long periods in diseased plant tissues.

Favourable conditions

- Spring session is responsible for the development of diseases.

PLANT PROTECTION BY PHENOLIC AND PHYTOALEXIN COMPOUND

- Phytoalexins are inducible secondary metabolites possessing antimicrobial activity toward phytopathogens .Since the diterpenoids momilactone A and B were identified as phytoalexins in rice leaves infected with blast fungus (*Magnaporthe oryzae*), many diterpenoid phytoalexins, including momilactones, phytocassanes and oryzalexins, have been identified from pathogen-infected rice The flavonoid sakuranetin is highly accumulated in rice leaves in response to blast infection and possesses strong antimicrobial activity against blast fungus, which suggests that it is an important phytoalexin in rice Although sakuranetin was identified as a phenolic phytoalexin, most rice phytoalexins are diterpenoid compounds, and research efforts have mainly focused on diterpenoid phytoalexins

- Until very recently, sakuranetin had been considered the only phenolic phytoalexin in rice. However, recent studies have shown that several phenylamides (amine-conjugated phenolic compounds) play a role as defense related agents exhibiting antimicrobial activity against rice pathogens This observation suggests that, along with sakuranetin, phenylamides are members of phenolic phytoalexins in rice. While the chemical nature and biosynthesis of diterpenoid phytoalexins have been extensively studied and reviewed there has been no comprehensive review of phenolic phytoalexins in rice. In this review, we summarize recent progress in rice phenolic phytoalexin research.

- As a physical barrier, the cell wall is important in plant defense against biotic and abiotic stresses. During the defense response to pathogen attacks and wounding, the cell wall is reinforced by deposition of cell wall biopolymers, such as callose and lignin, to prevent the entrance and propagation of invading pathogens. Wound-induced synthesis of phenylamides, such as feruloyltyramine and *p*-coumaroyltyramine, and their deposition in the cell wall are well known in wounded potato tissues, suggesting that they contribute to defensive biopolymer. In rice, tryptamine, serotonin and tryptamine derived phenylamides induced by *B. oryza* infection were reported to be deposited in the cell wall of lesion tissues. Treatment with the tryptamine biosynthesis inhibitor *S*-αFMT

suppressed the accumulation of tryptamine and its phenolic-conjugates and decreased the deposition of these materials in lesion tissues, which resulted in inhibitor treated leaves being susceptible to *B. oryzae* infection. This finding indicates that pathogen-induced rice phenylamides are involved in the defense response through reinforcement of the cell wall near infection sites in addition to their phytoalexin function.

- Allelochemicals are produced in plant tissues and are released into the environment, suppressing the growth and establishment of neighboring plants [. Allelopathic properties of diterpenoid phytoalexins, such as momilactones A and B, have been well established in rice. Momilactones are secreted from rice roots into the environment and play a role as allelochemicals . Phenolic acids, such as *p*-coumaric acid, ferulic acid and caffeic acid, were also isolated from the roots of allelopathic rice cultivars and were identified as allelochemicals. The phenolic acid moieties in phenylamide phytoalexins suggest that they likely act as allelochemicals in rice. In addition to UV and pathogen induced accumulation of phenylamide phytoalexins in rice leaves, CinTrp and BenTrp were isolated from rice roots without external stimulus [57]. A recent study also demonstrated that the phenylamide CinTyr isolated from rice acts as an allelochemical that inhibited root and hypocotyl growth of cress, barnyard grass, and red sprangletop [58]. This result suggests that phenylamide phytoalexins are potential allelochemicals in rice.

- Go to:

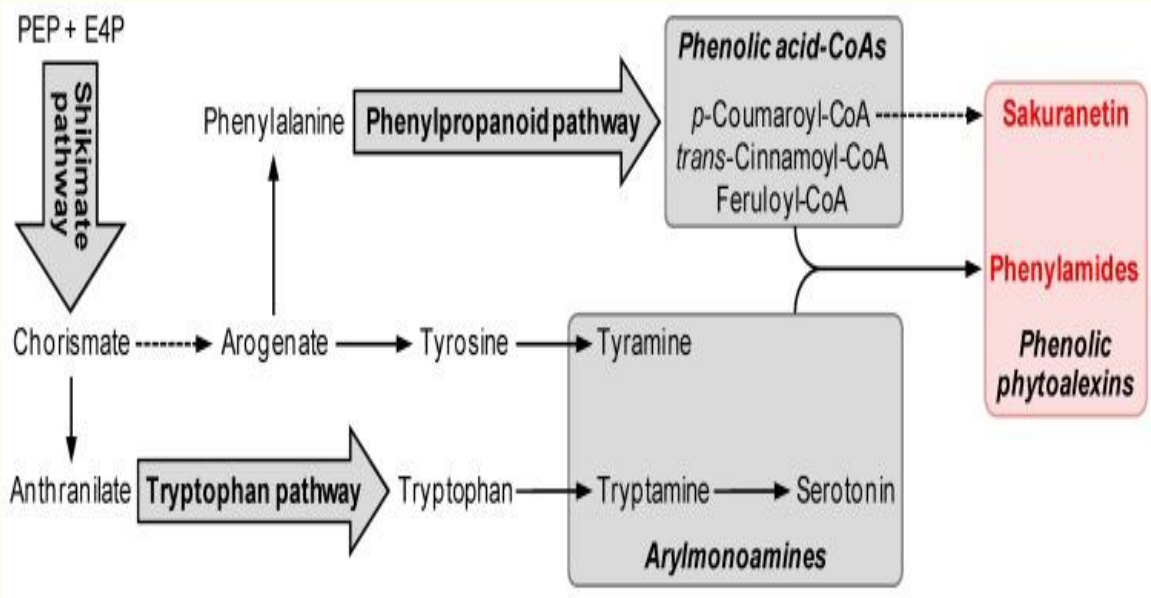
3. Biosynthesis of Rice Phenolic Phytoalexins

- During stress-induced production of sakuranetin and phenylamide phytoalexins, a series of metabolic pathways are potentially activated in rice tissues. The biosynthetic pathways implicated in rice phenolic phytoalexin synthesis include the shikimate pathway for aromatic L-amino acids (AAs) and the phenylpropanoid pathway for phenolic acid moieties in phenylamides and sakuranetin In plants, most genes for these pathway enzymes exist as multigene families, of which a set of genes are induced and implicated in biotic and abiotic stress-triggered synthesis of phenolic phytoalexins in rice Recently, functional studies of genes involved in phenolic phytoalexin synthesis have been performed with rice. Here, we summarize the induced biosynthetic pathways and genes for rice phenolic phytoalexin biosynthesis in response to various stresses, in particular UV irradiation and phytopathogen attack.

3.1. Shikimate and Phenylalanine Biosynthetic Pathway

- Aromatic AAs are building blocks for protein synthesis and serve as common precursors for plant secondary metabolites, such as phenolics and nitrogen containing compounds The shikimate pathway, an early biosynthetic pathway for aromatic AAs, is activated in plants under stress conditions The shikimate pathway synthesizes chorismate, a common intermediate for aromatic AAs, from phosphoenol pyruvate and erythrose 4-phosphate Activation of the shikimate pathway by pathogen attack was demonstrated with the metabolomic analysis of *M. oryzae* infected rice leaves. A recent transcriptomic analysis also showed that shikimate pathway genes were induced in rice leaves in response to UV

.A set of shikimate pathway genes including 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (*DAHPS*, Os07g42960), 3-dehydroquinate synthase (*DHQS*, Os09g36800), 3-dehydroquinate dehydratase/shikimate dehydrogenase (*DHQDT/SDH*, Os01g27750 and Os12g34874), shikimate kinase (*SK*, Os06g12150 and Os02g51410), and chorismate synthase (*CS*, Os03g14990), are immediately induced by UV treatment prior to the accumulation of sakuranetin and phenylamide phytoalexins, which implies the possible involvement of these genes in phytoalexin biosynthesis in rice. Chorismate is subsequently converted to L-phenylalanine (Phe) and L-tyrosine (Tyr) by chorismate mutase (CM), prephenate aminotransferase (PAT), and arogenate dehydratase (ADT) or arogenate dehydrogenase . Induction of *CM* (Os01g55870) and *ADT* (Os10g37980) were observed in rice leaves in response to UV treatment Direct evidence for a phytoalexin synthesis-related function of shikimate pathway genes and Phe and Tyr biosynthetic genes in rice has not yet been reported.



- Biotic and abiotic stress-induced metabolic pathways for phenolic phytoalexin biosynthesis in rice. The shikimate, phenylpropanoid and tryptophan pathways are coordinately activated by biotic and abiotic stresses to synthesize phenolic phytoalexins in rice. Phenolic acid-CoAs, such as *p*-coumaroyl-, *trans*-cinnamoyl- and feruloyl-CoAs, serve as intermediates in the formation of sakuranetin and phenylamide phytoalexins. Arylmonoamines, such as tryptamine, tyramine and serotonin, are conjugated with phenolic acid-CoAs to form phenylamide phytoalexins. Dashed arrows indicate multiple enzymatic steps. PEP; phosphoenol pyruvate, E4P; erythrose 4-phosphate.

Possible Questions:

PART-A (2Marks)

1. Discuss the importance of microbes in agriculture.
2. Explain in detail about the bacterial disease of cotton crop.

3.Comment on the symtoms, causative agent and control measures of bacterial disease in Paddy

4. Explain the role of phenolic compounds in plant protection

5.Describe the citrus canker disease.

Part B (8 Marks)

6. Illustrate the symtoms and control meatures of rice tungro disease in paddy.

*7.*Give a detailed account on Paddy crop.

8. Differentiate bacterial and fungal insectiside.

9.Give a detail account of bacterial disease of mango. Or

10.Discuss about phytoalexin.

S.no	Question	Opt A	OPT B	OPT C	OPT D	ANSWER
1	Citrus Canker is a _____ disease	Bacterial	Fungi	Virus	Protozoa	Bacterial
2	Citrus canker caused by____	Xanthomonas axonopodis	Xanthomonas pylori	Xanthomonas oryzae	*Pseudomonas syringae*	Xanthomonas axonopodis
3	Bacterial speck in tomato is caused by _____	Xanthomonas axonopodis	Xanthomonas pylori	Xanthomonas oryzae	*Pseudomonas syringae*	*Pseudomonas syringae*
4	The bacteria cannot survive in soil and dead plant parts	Xanthomonas axonopodis	Xanthomonas pylori	Xanthomonas oryzae	*Pseudomonas syringae*	Xanthomonas axonopodis
5	The bacterial _____they also may be present on volunteer tomato plants and on the surfaces of contaminated equipment	Speck pathogen	bacterial spot	bacterial canker	None of the above	Speck pathogen
6	*B*acterial speck, in tomato caused by___	Xanthomonas axonopodis	Xanthomonas pylori	Xanthomonas oryzae	*Pseudomonas syringae*	*Pseudomonas syringae*
7	. *B*acterial spot, in tomato caused by___	Xanthomonas axonopodis	Xanthomonas campestris	*Clavibacter michiganensis*	*Pseudomonas syringae*	Xanthomonas campestris
8	*B*acterial canker, in tomato caused by___	Xanthomonas axonopodis	Xanthomonas campestris	*Clavibacter michiganensis*	*Pseudomonas syringae*	*Clavibacter michiganensis*
9	Sesame leaf spot or Helminthosporiose or fungal blight in paddy is caused by	Helminthosporium oryzae	Xanthomonas campestris	*Clavibacter michiganensis*	*Pseudomonas syringae*	Helminthosporium oryzae
10	In paddy plant the fungal blight caused by_____	Helminthosporium oryzae	Xanthomonas campestris	*Clavibacter michiganensis*	*Pseudomonas syringae*	Helminthosporium oryzae
11	Sheath blight in rice caused by____	Helminthosporium oryzae	Xanthomonas campestris	*Rhizoctonia solani*	*Pseudomonas syringae*	*Rhizoctonia solani*
12	Sheath rot in rice caused by____	Xanthomonas axonopodis	Xanthomonas pylori	Sarocladium oryzae	*Pseudomonas syringae*	Sarocladium oryzae
13	_____ is restricted to black cotton soils with pH 7.6-8.00	Wilt	Rust	*Vascular*	*all the above*	Wilt
14	Wilt is restricted to ____ cotton soils with pH 7.6-8.00	red	brown	*black*	*yellow*	black

15	Wilt is restricted to black cotton soils with _____pH	7.6-8.00	8.00-8.5	*8.5-9.00*	*9.00-9.5*	7.6-8.00
16	Boll Rot disease caused by_____	*Colletotrichum capsici*	Aspergillus flavus	Rhizopus nigricans	*all the above*	All the above
17	Leaf Spot or Blight Disease in cotton plant caused by_____	*Colletotrichum capsici*	Aspergillus flavus	Rhizopus nigricans	Alternaria macrospora Zimm	Alternaria macrospora Zimm
18	This disease also reported due to soil deficient in magnesium	Sheath rot	sheath blight	Reddening and Lalya disease	Wilt	Reddening and Lalya disease
19	Reddening and Lalya disease also reported due to soil deficient in	Magnesium	Iron	Sulphur	*Phosphorus*	Magnesium
20	Crops like cotton, tomato, tobacco are extremely sensitive to 2, 4-D which can cause harmful damage to crops (cotton).	Reddening and Lalya disease	2,4 D Injury Disease	Sheath rot	sheath blight	2,4 D Injury Disease
21	Tobacco Streak Virus in cotton plant otherwise called____	liar virus	tobacco virus	both a&b	None of the above	liar virus
22	Liar virus also known as____	Peanut virus	tobacco virus	Tobacco streak virus	Tobacco mosaic virus	Tobacco streak virus
23	Rice blast disease also known as____	Rice fever	Leaf blight	brown spot	sheath blight	Rice fever
24	Rice fever disease also known as____	Rotten neck	Leaf blight	brown spot	sheath blight	Rotten neck
25	Bacterial wilt is caused by a soil-borne bacterium named as___	*Ralstonia solanacearum*	Aspergillus flavus	Rhizopus nigricans	Alternaria macrospora Zimm	*Ralstonia solanacearum*
26	Bacterial wilt is caused by a soil-borne bacterium named *Ralstonia solanacearum* earlier known as _____	Aspergillus flavus	Rhizopus nigricans	Alternaria macrospora Zimm	*Pseudomonas solanacearum*	*Pseudomonas solanacearum*
27	Biosynthesis of phenolic compounds	Citric acid	Shikimic acid	Butric acid	Sulphuric acid	Shikimic acid

	in plants by ____ pathway.					
28	In phenolic Shikimic acid pathway converts simple carbohydrate in to____	aromatic amino acid	aldehydes	alcohols	None of the above	aromatic amino acid
29	Biosynthesis of phenolic Shikimic acid pathway is most common in ____	Animal	microbes	Plants	all the above	Plants
30	Plant phenolics are derived from cinnamic acid formed from phenylalanine by___	Phenylalanine ammonia lyase	ammonia lyase	lyase	all the above	Phenylalanine ammonia lyase
31	PAL(Phenylalanine ammonia lyase) activity induced by___	low light	high temperature	low temperature	high light	high light
32	Insect roll leave can prevent by____ phenolic compound	Furoanocoumarins	Phenylalanine	Malonic acid	None of the above	Furoanocoumarins
33	give a suitable example for plant flavanoids	anthocyanin	flavones	flavonols	all the above	all the above
34	Condensed tannin are polymerized____	phenols	flavonoids	lignin	alcohol	flavonoids
35	intermediate product of shikimic acid pathway	Furoanocoumarins	Phenylalanine	Malonic acid	cinnamic acid	cinnamic acid
36	second most abundant compound in plants	phenols	flavonoids	lignin	alcohol	lignin
37	Hydrolyzable tannins made up of____	acids	sugars	acid and sugars	alcohols	acid and sugars
38	Phytoalexins produced in plants act as ____to the attacking organism.	toxins	sugars	enzymes	antibiotics	toxins
39	systemic acquired resistance (SAR), involves communication of the damaged tissue with the rest of the plant using plant	sulphuric acid	Jasmonic acid	Shikimic acid	Citric acid	Jasmonic acid

	hormones such as					
40	_____is a phytoalexin compound isolated from garlic	phenols	ethelene	Allixin	Polyphenol	Allixin
41	_____ is a phytoalexin found in the papaya fruit.	phenols	ethelene	Danielone	Allixin	Danielone
42	High levels of _____ in some woods can explain their natural preservation against rot	polyphenols	phenols	both a&b	None of the above	polyphenols
43	The phytoalexin compound are produced in Eucalyptus sideroxylon in case of pathogens attacks.	ethelene	Danielone	Allixin	Stilbenes	Stilbenes
44	The phytoalexin compound which isolated from soy bean____	Glyceolin	Danielone	Allixin	Stilbenes	Glyceolin
45	The phytoalexin from grapevine has anti aging, anticarcarcinogenic, anti-inflammatory and anti oxidant properties.	Glyceolin	resveratrol	Danielone	Stilbenes	resveratrol
46	Abnormal sesquiterpinoid induced in sweet potato tissue infected with black rot fungus *Ceratocystis fimbriata*.	resveratrol	Danielone	Stilbenes	Ipomoeamarone	Ipomoeamarone
47	Name the phytoalexin produced by peapods inoculated with *Monilia fructicola.*	Glyceolin	Danielone	pisatin	Stilbenes	pisatin
48	Parasporal body possesses insecticidal	B.thuringiensis	B.Subtilis	B.megaterium	*Beauveria bassiana*	B.thuringiensis

	properties					
49	Parasporal body comprises of crystals and tightly packed with proteins called___	endotoxin	protoxin	exotoxin	both a&b	both a&b
50	Parasporal body comprises of crystals and tightly packed withproteins is converted in to active toxin requires___ environment.	acidic	alkali	neutral	None of the above	alkali
51	Parasporal body comprises of crystals and tightly packed withproteins is converted in to active toxin requires alkali environment ranges from	7.5-8	7.5-8.5	7-8.1	8-7	7.5-8
52	B.thuringienis protoxin molecular weght	131 kDa	130 kDa	132 kDa	133 kDa	130 kDa
53	B.thuringienis active toxin molecular weght	68kDa	67kDa	66kDa	65kDa	68kDa
54	Fungi used as a biological insecticide to control a number of pests such as termites, whiteflies and different beetles	*Beauveria bassiana*	*Hirsutella citriformis*	*Hirsutella abeitina*	Hirsutella fusiformis	*Beauveria bassiana*
55	fungi which used control leafhoppers and plant hoppers	*Hirsutella citriformis*	*Beauveria bassiana*	B.Subtilis	B.megateri um	*Hirsutella citriformis*
56	Which of the following microbe is widely used in the removal of industrial waste	Trichoderma sp	Aspergillus niger	Pseudomona s putida	Bacillus	Aspergillus niger
57	_____are mixed cultures of naturally occuring beneficial microbes used to degrade	Enhanced microorganis ms	Efficient microorgani sms	Effective microorganis ms	Good microorgan isms	Effective microorganis ms

	contaminants, increase quality of soil					
58	____is a key ingredient in organic farming	compost	urea	Pesticide	fungicide	compost
59	oldest form of waste treatment	Burning	Shredding	Vermicompostin	Landfill	Landfill
60	The process of composting using earthworms is called as	Degradation	Vermicomposting	Bioaugumentation	organic treatment	Vermicomposting

UNIT – IV

Biological nitrogen fixation - symbiotic and non-symbiotic microorganisms, root nodule formation, nitrogen fixers, hydrogenase, Nitrogenase, *Nif* gene regulation. Biochemistry of nitrogen fixation, Rhizosphere- R: S ratio, Interaction of microbes with plants. Bioconversion of agricultural wastes. Genetically Modified organisms and crops.

Biological Nitrogen Fixation: Biological nitrogen fixation (BNF), discovered by Beijerinck in 1901 (Beijerinck 1901), is carried out by a specialized group of prokaryotes. These organisms utilize the enzyme nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Plants can readily assimilate NH_3 to produce the aforementioned nitrogenous biomolecules. These prokaryotes include aquatic organisms, such as cyanobacteria, free-living soil bacteria, such as *Azotobacter*, bacteria that form associative relationships with plants, such as *Azospirillum*, and most importantly, bacteria, such as *Rhizobium*and *Bradyrhizobium*, that form symbioses with legumes

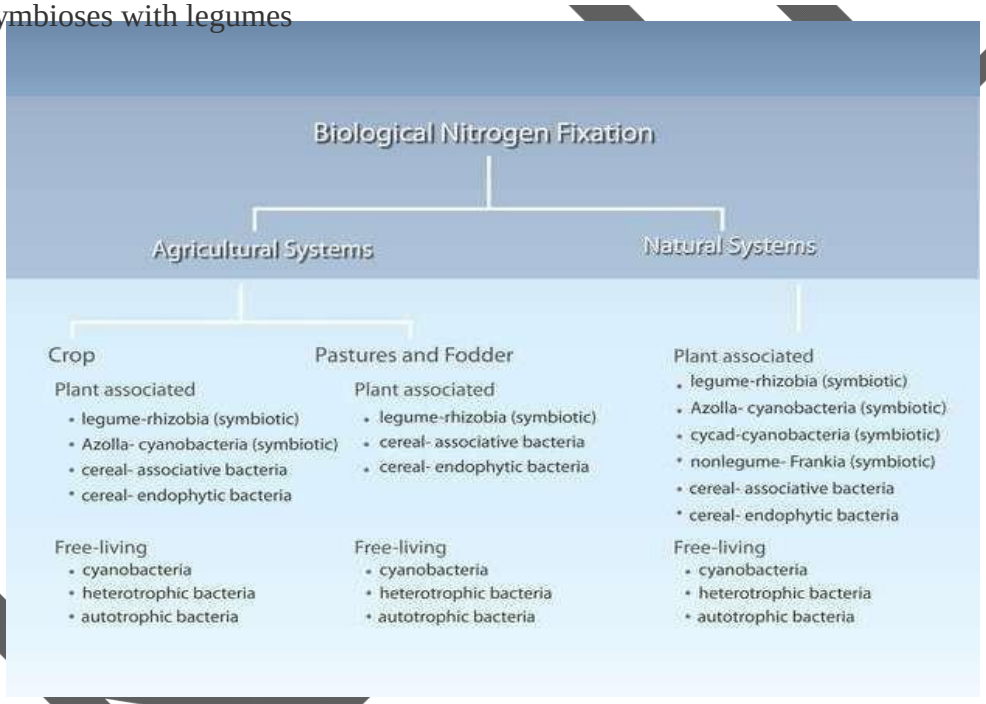


Table 1. Nitrogen-fixing organisms found in agricultural and natural systems.

Microorganisms that fix nitrogen require 16 moles of adenosine triphosphate (ATP) to reduce each mole of nitrogen. These organisms obtain this energy by oxidizing organic molecules. Non-photosynthetic free-living microorganisms must obtain these molecules from other organisms, while photosynthetic microorganisms, such as cyanobacteria, use sugars produced by photosynthesis. Associative and symbiotic nitrogen-fixing microorganis n s obtain these compounds from their host plants' rhizospheres

Two kinds of nitrogen fixers are recognized: free-living (non-symbiotic) bacteria, including the cyanobacteria (or blue-green algae) *Anabaena* and *Nostoc* and such genera as *Azotobacter*,

Beijerinckia, and *Clostridium*; and mutualistic (symbiotic) bacteria such

as *Rhizobium*, associated with leguminous plants, and *Spirillum lipoferum*, associated with cereal grasses.

The symbiotic nitrogen-fixing bacteria invade the root hairs of host plants, where they multiply and stimulate formation of root nodules, enlargements of plant cells and bacteria in intimate association. Within the nodules the bacteria convert free nitrogen to nitrates, which the host plant utilizes for its development. To insure sufficient nodule formation and optimum growth of legumes (*e.g.*, alfalfa, beans, clovers, peas, soybeans), seeds are usually inoculated with commercial cultures of appropriate *Rhizobium* species, especially in soils poor or lacking in the required bacterium.

Nitrogen Fixation by Free-Living Heterotrophs: Many heterotrophic bacteria live in the soil and fix significant levels of nitrogen without the direct interaction with other organisms. Examples of this type of nitrogen-fixing bacteria include species of *Azotobacter*, *Bacillus*, *Clostridium*, and *Klebsiella*. As previously noted, these organisms must find their own source of energy, typically by oxidizing organic molecules released by other organisms or from decomposition. There are some free-living organisms that have chemolithotrophic capabilities and can thereby utilize inorganic compounds as a source of energy. Because nitrogenase can be inhibited by oxygen, free-living organisms behave as anaerobes or microaerophiles while fixing nitrogen. Because of the scarcity of suitable carbon and energy sources for these organisms, their contribution to global nitrogen fixation rates is generally considered minor. However, a recent study in Australia of an intensive wheat rotation farming system demonstrated that free-living microorganisms contributed 20 kilograms per hectare per year to the long-term nitrogen needs of this cropping system (30-50% of the total needs. Maintaining wheat stubble and reduced tillage in this system provided the necessary high-carbon, low-nitrogen environment to optimize activity of the free-living organisms.

Associative Nitrogen Fixation: Species of *Azospirillum* are able to form close associations with several members of the *Poaceae* (grasses), including agronomically important cereal crops, such as rice, wheat, corn, oats, and barley. These bacteria fix appreciable amounts of nitrogen within the rhizosphere of the host plants. Efficiencies of 52 mg N_2 g^{-1} malate have been reported (Stephan *et al*. 1979). The level of nitrogen fixation is determined by several factors, including soil temperature (*Azospirillum* species thrive in more temperate and/or tropical environments), the ability of the host plant to provide a rhizosphere environment low in oxygen pressure, the availability of host photosynthates for the bacteria, the competitiveness of the bacteria, and the efficiency of nitrogenase

Symbiotic Nitrogen Fixation: Many microorganisms fix nitrogen symbiotically by partnering with a host plant. The plant provides sugars from photosynthesis that are utilized by the nitrogen-fixing microorganism for the energy it needs for nitrogen fixation. In exchange for these carbon sources, the microbe provides fixed nitrogen to the host plant for its growth.

Example of this type of nitrogen fixation is the water fern *Azolla*'s symbiosis with a cyanobacterium *Anabaena azollae*. *Anabaena* colonizes cavities formed at the base of *Azolla* fronds. There the cyanobacteria fix significant amounts of nitrogen in specialized cells called heterocysts. This symbiosis has been used for at least 1000 years as a biofertilizer in

wetland paddies in Southeast Asia. Rice paddies are typically covered with *Azolla*–blooms that fix up to 600 Kg N ha^{-1} yr^{-1} during the growing season. Another example is the symbiosis between actinorhizal trees and shrubs, such as Alder (*Alnus* sp.), with the actinomycete *Frankia*. These plants are native to North America and tend to thrive in nitrogen-poor environments. In many areas they are the most common non-legume nitrogen fixers and are often the pioneer species in successional plant communities. Actinorhizal plants are found in many ecosystems including alpine, xeric, chapparal, forest, glacial till, riparian, coastal dune, and arctic tundra environments.

Even though the symbiotic partners described above play an important role in the worldwide ecology of nitrogen fixation, by far the most important nitrogen-fixing symbiotic associations are the relationships between legumes and *Rhizobium* and *Bradyrhizobium* bacteria. Important legumes used in agricultural systems include alfalfa, beans, clover, cowpeas, lupines, peanut, soybean, and vetches. Of the legumes in agricultural production, soybeans are grown on 50% of the global area devoted to legumes, and represent 68% of the total global legume production.

2. Symbiotic micro organisms

Bacterial Symbiosis: Bacteria form symbiotic relationships with many organisms, including humans. One example is the bacteria that live inside the human digestive system. These microbes break down food and produce vitamins that humans need. In return, the bacteria benefit from the stable environment inside the intestines. Bacteria also colonize human skin. The bacteria obtain nutrients from the surface of the skin, while providing people with protection against more dangerous microbes.

Symbiotic bacteria also live in nodules on the roots of bean plants. These bacteria convert nitrogen gas into a form that the plants can use. In return, the plants provide the bacteria with a safe place to live. In some cases, the symbiotic relationship is more strong. One particular roundworm has a bacterium living inside it. The roundworm infects and kills insects, using a toxin produced by the bacterium. This is an example of mutualism, because the roundworm and bacterium need each other to survive.

Fungi and Plants: Fungi and plants form mutually-beneficial relationships called mycorrhizal associations. The fungi increase the absorption of water and nutrients by the plants, and benefit from the compounds produced by the plants during photosynthesis. The fungus also protects the roots from diseases. Some fungi form extensive networks beneath the ground, and have been known to transport nutrients between plants and trees in different locations.

Fungi and plant roots form two different kinds of associations. In one type, the fungus grows outside the roots as a thick mat, or between certain cells in the root. The fungus, however, never enters any of the plant cells. With the other type of association, the fungus actually penetrates the cell walls of the roots. They break through the cell wall, but not the inner plasma membrane. In both types, the fungus extends its filaments outward to collect nutrients and water from the soil, which are in turn passed onto the plant.

Lichens: Fungi and Algae

Lichens are an example of a symbiotic relationship between two microbes, fungi and algae. So far, around 25,000 lichens have been identified. They grow on rocks and tree trunks, with colors ranging from pale whitish green to bright red and orange. The lichens grow in several forms: thin and crusty coverings; small branching strands; or flat, leaf-like structures. They are usually the first plants to grow in the cold and dry habitats that they favor.

In this mutually-beneficial relationship, the fungus forms the body of the lichen — the thallus. This structure attaches to the surface of a rock or tree. The fungal cells absorb water and nutrients from the surrounding environment. Algal cells grow inside the cells of the fungus. The algal cells convert sunlight to chemical energy through photosynthesis. This process benefits the fungus. In return, the algal cells are protected from the environment.

Protists : Certain protists and algae form a symbiotic relationship known as living sands. This type of association occurs in tropical and semitropical seas, and appears as green, orange, brown or red deposits containing calcium carbonate. Living sands were used in the construction of the Egyptian pyramids. Many different types of algae combine with their protist hosts. Without the algae, the protists cannot survive very long. Similar to living sands, some protists extract chloroplasts from diatoms, a type of algae. The chloroplasts provide the protists with the ability to convert sunlight to chemical energy through photosynthesis. Eventually, the chloroplasts break down and stop functioning.

An even better-known example of symbiotic protists are the ones that live in the guts of termites. These microbes break down cellulose in the wood particles that termites eat. This enables the termite to obtain nutrition from the wood. Without the help of the protists, the termite would not be able to digest the wood. In this case, the protist is called an endosymbiont, which means it lives inside its host, the termite.

Early Eukaryotes: Some scientists believe that early in the history of the planet, different types of microbes joined together to form a new type of organism. At the time, certain bacteria had the ability to convert sunlight to chemical energy, or generate chemical energy from oxygen. These microbes were engulfed by larger bacteria, forming a microbial symbiosis. The host cell protected the smaller microbe inside, while benefitting from the skills of its new partner.

In the beginning, the two bacteria could still function separately. Eventually, the microbes living inside lost the ability to survive on their own, and they became specialized components of the host cells. These structures later became the mitochondria and chloroplasts of eukaryotic cells. Mitochondria generate energy using oxygen, and chloroplasts convert sunlight into chemical energy in plant cells. Supporting this theory is the fact that mitochondria and chloroplasts both have their own DNA, separate than that found in the nucleus of the cell.

3. Non-symbiotic Nitrogen fixers

Azospirillum : Eighty percent (80 %) of the atmosphere is nitrogen gas (N2). Unfortunately N2 is unusable by most living organisms. Plants, animals, and microorganisms can die of nitrogen

deficiency, if surrounded by N2 they cannot use. All organisms use the ammonia (NH3) form of nitrogen to manufacture amino acids, proteins, nucleic acids, and other nitrogen-containing components necessary for life. Biological nitrogen fixation (BNF) process changes inert N2 to useful NH3. This process is mediated in nature only by bacteria and certain species of actinomycetes. In the free-living system, plants gain benefit when the bacteria die and release nitrogen to the environment, or when the bacteria are loosely associated with the roots of plants. In legumes and a few other plants, the bacteria live in small club-like growths on the roots called nodules. Within these nodules, N2 fixation occurs, and the NH3 produced is directly absorbed by the plant. Nitrogen fixation by legumes is a close/symbiotic relationship between a Rhizobium bacterium and a legume host plant. Azospirillum as a ‑biofertilizer‖ is particularly important in agricultural systems where fertilizer inputs are either impractical (rangelands), undesirable (organic farming), or not possible (subsistence agriculture). Experiments on inoculation of crops with Azospirillum or other diazotrophs resulted in enhanced plant growth or nitrogen content under environmental conditions, improve nutrient assimilation, alter root size and function. Numerous studies have shown greater N2 fixation activities in inoculated plants than in uninoculated controls. The report shows higher N2 fixation rates were observed near or at flowering stage particularly under conditions of high temperature and soil moisture. In addition to N2 fixation, inoculation with Azospirillum results in the following benefits:

1. Promotion of root hair development and branching;
2. Increased uptake of N, P, K and microelements;
3. Improved water status of plants and,
4. Increased dry matter accumulation and grain yield.

Inoculated plants when examined under the electron microscopes revealed invasion of the cortical layer. Azospirillum species are described as Gram negative, rod-shaped, 1mm in diameter, very motile. Cells are about 1.0 um x 3.5 mm in size single flagellum when grown in MPSS broth while lateral flagella when grown on MPSS agar at 30 ºC. They also form wrinkled, dark pink colonies when grown on MPSS agar. A formation of a white veil or bacteria band, is visible when inoculated into an Nfb and Dobereiner's liquid medium. Azospirillum utilizes glucose, lactate, succinate, fructose, malate, pyruvate, fumarate, as carbon source, reduced nitrate and does not require biotin. The N source used by Azospirillum for their growth**: 1.** Ammonium **2.**Nitrate **3.**Amino acids **4.**Elemental N. *Azospirillum spp*. are highly adaptable, being able to grow under:

1.Anaerobic conditions (nitrate used as eletron acceptor) **2.**Microaerobic (elemental or ammonia used as N source) **3.**Fully aerobic conditions (ammonia, nitrate, amino acid or combined N only) Preliminary field experiments in Batangas, Pangasinan, Laguna, Bulacan and Cagayan Valley showed when BIO-N inoculated corn produce a comparatively high yield in the presence of 1/3 to 2/3 of the required N fertilizer. In most of the test sites, the inoculated but unfertilized plots gave rise to consistently and significantly taller and greener plants than the uninoculated unfertilized control, particularly at sixty days after planting.

4. Legume Nodule Formation

The *Rhizobium* or *Bradyrhizobium* bacteria colonize the host plant's root system and cause the roots to form nodules to house the bacteria. The bacteria then begin to fix the nitrogen required by the plant. Access to the fixed nitrogen allows the plant to produce leaves fortified with nitrogen that can be recycled throughout the plant. This allows the plant to increase photosynthetic capacity, which in turn yields nitrogen-rich seed. The consequences of legumes not being nodulated can be quite dramatic, especially when the plants are grown in nitrogen-poor soil. The resulting plants are typically chlorotic, low in nitrogen content, and yield very little seed.

The nodulation process illustrates an orchestrated interaction between the bacteria and host plant. The process begins when the rhizobia are attracted to flavonoids released by the host legume's roots. For legumes like alfalfa, clover, and soybeans (others like lupines and peanuts form nodules in other ways) the bacteria then begin to attach themselves to extensions of root epidermal cells called root hairs. The attachment process is actually a two-step process where the bacteria first attach using a Ca^{2+} - binding protein called rhicadhesin. After the bacteria accumulate and anchor themselves to the root hair surface, a firmer attachment that involves lectins and/or cellulose firbrils and fimbriae produced by the host plant and bacteria, respectively.

The host legume then senses chemicals produced by the rhizobia called Nod factors that cause the colonized root hairs to curl and form what is called a shepherd's crook. Then rhizobia penetrate the root hairs and typically form a tubular structure called an infection thread. Once the bacteria reach the root itself, they stimulate cortical cell divisions that lead to the formation of a nodule. As the nodule begins to form, the bacteria become surrounded by a plant-derived membrane and are released inside plant cells forming the nodule. The bacteria subsequently lose their cell walls and undergo a profound change in cell morphology to form large, irregularly shaped branching cells called bacteroids. They then are entirely dependent on the host plant for their energy needs. In return, the bacteria fix nitrogen for the plant.

The interaction between the bacteria and host legume is so intricate that a particular *Rhizobium* or *Bradyrhizobium* will only nodulate a select number of plant genera. For example, *Rhizobium melilotii* will only nodulate alfalfa, while *Rhizobium leguminosarum biovar trifolii* will only nodulate clover (*Trifolium*). This host specificity is referred to cross inoculation group cell signaling between the bacteria and the legume host. The aforementioned Nod factors have been identified as lipochition oligosaccharides. Variations in the structures of these oligosaccharides determine the host specificity for the bacterium.

Nitrogen is an essential nutrient for plant growth and development but is unavailable in its most prevalent form as atmospheric nitrogen. Plants instead depend upon combined, or fixed, forms of nitrogen, such as ammonia and nitrate. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers. Use of these fertilizers has led to worldwide, ecological problems, such as the formation of coastal dead zones. Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants. It is a critical component of many aquatic, as well as terrestrial ecosystems across our biosphere.

5. Nitrogen fixers and Hydrogenase enzyme: zotobacter possesses an active hydrogenase, the enzyme which catalyzes the oxidation of hydrogen. Some evidence has been uncovered which suggests that the occurrence of this enzyme is correlated with nitrogen fixation by this organism. If true, this has great significance for studies of the mechanism of biological nitrogen fixation. A detailed investigation was accordingly undertaken and it was measured the hydrogenase activity of Azotobacter cells grown or maintained under conditions which would vary the rate or extent of the nitrogen-fixing reaction. These included (a) the use of various forms of combined nitrogen; (b) variation in the level of combined nitrogen; (c) adaptation of cells to combined nitrogen; (d) growing cells in the presence of hydrogen and absence of free nitrogen. The general methods have been described in earlier reports; any necessary details will be furnished in the text.

Effect of pH of Medium on Hydrogenase in Axotobacter: In the initial experiments with combined nitrogen (2) ammonium phosphate usually served as a source of this element. During growth a pronounced decrease in the pH occurred because of selective utilization of the ammonium ion. however, it is the presence of combined nitrogen in the medium and not the alteration of pH which is accompanied by a decrease in hydrogenase. The data demonstrate that the hydrogenase con- tent of Axotobacter drops in the presence of ammonium ion, whet,her the pH rises, falls, or remains constant.

Effect of Combined Nitrogen on Hydrogenase in Various Species of Azotobacter-Cultures of *Axotobacter vinelandii, A. agile*, and *,4. chroococcum* were grown on Burk's N-free agar medium and on the same medium plus combined nitrogen in various forms (50 mg. of N per 100 ml.). Table II gives the results of these experiments. As noted previously with *A. vinelandii* (2), NHk-N is much more effective in reducing the hydrogenase content than is NOS-N, whereas glutamate N has little if any effect.

6. Nitrogenase

Nitrogenase and its metalloclusters : Diazotrophic organisms can fix N2 because they produce an enzyme called nitrogenase. Most N2 fixing organisms studied so far produce a Molybdenum-containing 2 nitrogenase. In addition, some organisms have –alternative| systems that produce a Vanadium-containing nitrogenase and/or an iron-only nitrogenase. Among these three classes of nitrogenase, the Mo-containing nitrogenase is the most prevalent and the best characterized. Mo-containing nitrogenases are composed of two oxygen – sensitive components designated the MoFe protein and the Fe protein. Together under the ideal conditions, they catalyze the following reaction:

$$N_2 + 8H^+ + 8e^- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi$$

7. Nif gene regulation

Fig:3

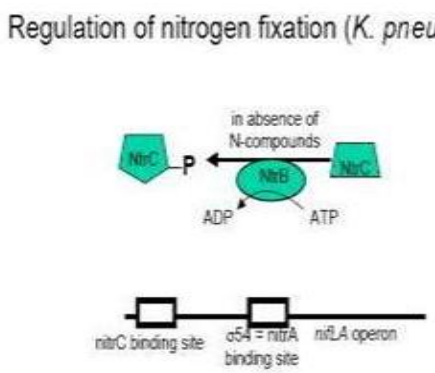


Fig:4

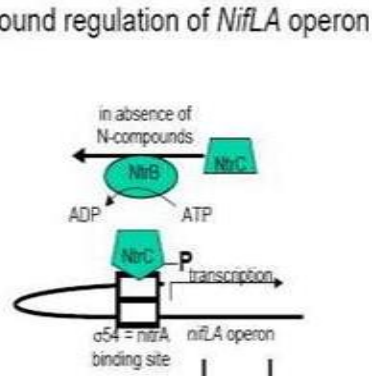


Fig:5

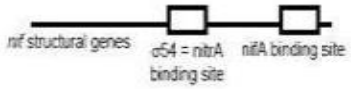
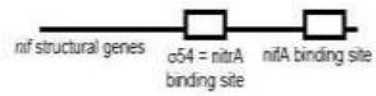


Fig:6



8. Biochemistry of Nitrogen fixation: To understand the biosynthesis of the iron-molybdenum cofactor of nitrogenase **(FeMo-co; Figure 7**) and to improve biological hydrogen production by altering the catalytic properties of nitrogenase. Our future research plans include applying the knowledge obtained through basic science studies of nitrogenase biogenesis to engineer active nitrogenase in eukaryotes.

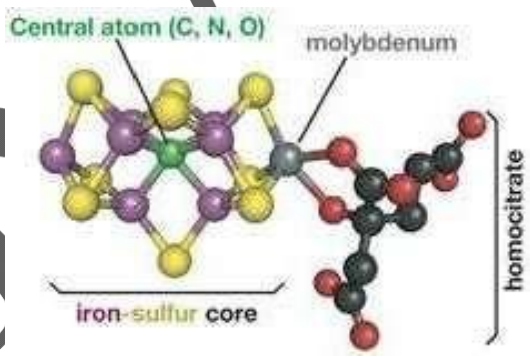


Fig: 7 Structure of the nitrogenase enzyme complex

FeMo-co, located at the active site of the nitrogenase enzyme is utimately responsible for biological nitrogen fixation, a process that transforms inert atmospheric N2 into a form that can be metabolized by organisms **(Figure 8).** Although only a small group of bacteria and archea are able to fix nitrogen, this essential natural process supports life on earth. Due to its great commercial (agricultural) significance, nitrogenase has been subjected to extensive biochemical, genetic, and structural analyses. Understanding the details of FeMo-co synthesis and nitrogenase assembly could, in the long term, improve the agronomical applications of biological nitrogen fixation.

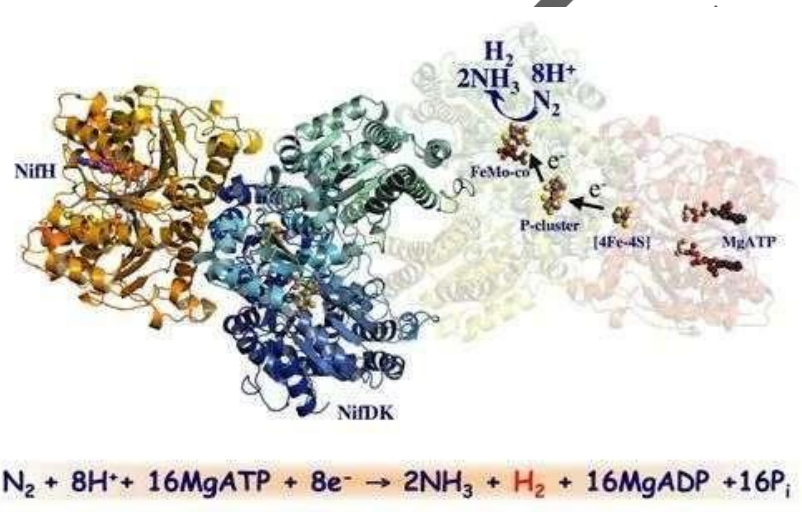


Fig:8 Structure of the Nitrogenase enzyme complex

9.Rhizosphere-R:S ratio

The root system of higher plants is associated not only with soil environment composed of inorganic and organic matter, but also with a vast community of metabolically active microorganisms. As living plants create a unique habitat around the roots, the microbial population on and around the roots is considerably higher than that of root free soil environment and the differences may be both quantitative and qualitative.

1. **Rhizosphere:** It is the zone/region of soil immediately surrounding the plant roots together with root surfaces, or it is the region where soil and plant roots make contact, or it is the soil region subjected to influence of plant roots and characterized by increased microbial.

2. **Rhizoplane:** Root surface along with the closely adhering soil particles is termed as rhizoplane.

Microorganisms in the Rhizosphere and Rhizosphere Effect

The rhizosphere region is a highly favorable habitat for the proliferation, activity and metabolism of numerous microorganisms. The rhizosphere microflora can be enumerated intensively by microscopic, cultural and biochemical techniques. Microscopic techniques reveal the types of organisms present and their physical association with the outer root tissue surface / root hairs. The cultural technique most commonly followed is "serial dilution and plate count method" which reveal the quantitative and qualitative population of microflora. At the same time, a cultural method shows the selective enhancement of certain categories of bacteria. The biochemical techniques used are designed to measure a specific change brought about by the plant or by the microflora. The rhizosphere effect on most commonly found microorganisms viz. bacteria, actinomycetes, fungi, algae and protozoa is being discussed herewith in the following paragraphs.

A. Bacteria: The greater rhizosphere effect is observed with bacteria (R: S values ranging from 10-20 or more) than with actinomycetes and fungi. Gram-negative, rod shaped, non-sporulating bacteria which respond to root exudates are predominant in the rhizosphere*(Pseudomonas, Agrobacterium).* While Gram-positive, rods, Cocci and aerobic spore forming *(Bacillus, Clostridium)* are comparatively rare in the rhizosphere. The most common genera of bacteria are: *Pseudomonas, Arthrobacter, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas, Micrococcus* and others have been reported to be either abundant or sparse in the rhizosphere. From the agronomic point of view, the abundance of nitrogen fixing and phosphate solubilizing bacteria in the rhizosphere assumes a great importance. The aerobic bacteria are relatively less in the rhizosphere because of the reduced oxygen levels due to root respiration. The bacterial population in the rhizosphere is enormous in the ranging form 10^8 to 10^9 per gram of rhizosphere soil. They cover about 4-10% of the total root area occurring profusely on the root hair region and rarely in the root tips. There is predominance of amino acids and growth factors required by bacteria, are readily provided by the root exudates in the region of rhizosphere.

B. Fungi: In contrast to their effects on bacteria, plant roots do not alter / enhance the total count of fungi in the rhizosphere. However, rhizosphere effect is selective and significant on specific fungal genera *(Fusarium, Verticillium, Aspergillus* and *Penicillium)* which are stimulated. The R:S ratio of fungal population is believed to be narrow in most of the plants, usually not exceeding to 10. The soil / serial dilution and plating technique used for the enumeration of rhizosphere fungi may often give erratic results as most of the spore formers produce abundant colonies in culture media giving a wrong picture / estimate *(eg Aspergilli* and *Penicillia).* In fact the mycelial forms are more dominant in the field. The zoospore / forming lower fungi such as *Phytophthora, Pythium, Aphanomyces* are strongly attracted to the roots in response to particular chemical compounds excreted by the roots and cause diseases under favorable conditions. Several fungi *eg Gibberella* and *fujikurio*produces *phytohormones* and influence the plant growth.

C. Actinomycetes, Protozoa and Algae: Stimulation of actinomycetes in the rhizosphere has not been studied in much detail so far. It is generally understood that the actinomycetes are less stimulated in the rhizosphere than bacteria. However, when antagonistic actinomycetes increase in number they suppress bacteria. Actinomycetes may also increase in number when antibacterial agents are sprayed on the crop. Among the actinomycete, the phosphate solublizers (eg.*Nocardia, Streptomyces)* have a dominant role to play.

As rule actinomycetes, protozoa and algae are not significantly influenced by their proximity to the plant roots and their R: S ratios rarely exceed 2 to 3: 1 and around roots of plants, R: S ratio for these microorganisms may go to high. Because of large bacterial community, an increase in the number or activity of protozoa is expected in the rhizosphere. Flagellates and amoebae are dominant and ciliates are rare in the region.

Table:3 Factors Responsible for the Development of the Soil-Plant Root Rhizosphere.

Release of soluble organic compounds by plant roots
Sloughed off root cell debris and dying root hairs
Plant root cell lysis
Higher concentration of carbon dioxide
Lower concentration of oxygen
Lower concentration of nutrient ions
Partial desiccation of soil due to absorption of water by roots

10. Interaction of microbes with plants: Plants are in contact with diverse microbes blown by the wind, delivered via the water cycle, and recruited to their roots and leaves from the soil. Many of these microbes are unable to start their life cycle in association with a living plant. Others are potential pathogens, potential symbionts, or harmless commensals. The ultimate outcome of plant-microbe interactions is tuned by host and microbe genotypes and by the environmental context. All land plants grow in intimate association with complex microbial communities. These attach to, and inhabit, both the roots (rhizosphere) and aboveground organs (phyllosphere) as epiphytes or endophytes. Plant-derived exudates and secreted secondary metabolites are implicated in encouraging specific microbial colonization. Host plants often rely on the associated microbiome for one or more critical nutrients, such as fixed nitrogen. The plant, in turn, can provide fixed atmospheric carbon to some members of the microbiome, thus acting as a carbon sequestration niche.

Plant interactions with microbes are important in the context of plant health and global food security. Yield losses due to microbial pathogens and pests can be up to 30 percent worldwide, and much of this loss takes place after the freshwater input required to grow a crop. Thus, if we could better combat microbial infection of plants via rational deployment of the plant immune system, we could save significant amounts of water and spare significant acreage from the plow.

Additionally, if we could better understand and deploy the plant immune system, we could diminish or eliminate the use of chemicals in the control of plant disease.

The Plant Immune System: Plants express a two-tiered immune system that has analogies to the mammalian innate immune system. Microbes express microbial-associated molecular patterns (MAMPs) on their surfaces. MAMPs can be sensed specifically by plant cell-surface pattern-recognition receptors (PRRs). Plant PRRs described to date are cell-surface receptors featuring an ectodomain, most commonly a leucine-rich repeat (LRR), a transmembrane domain, and a cytosolic kinase domain. Plant PRRs are analogous to the familiar TLR receptors of the animal innate immune system. MAMP recognition leads to signal transduction and transcriptional reprogramming, resulting in the initiation of MAMP-triggered immunity (MTI). MTI is sufficient to halt the growth of most microbes. Hence, most microbes are not pathogens.

The Plant Microbiome: All land plants grow in intimate association with a complex root microbiota that is distinct from the microbial community present in bulk soil. These interactions are driven by the influence of root physiology and metabolism, which influence the rhizoplane (the 1 mm surrounding the root) environment through adjusting the soil pH changing soil structure and oxygen availability, producing antimicrobials and quorum-sensing mimics that manipulate microbial communication, providing an energy source in the form of dead root material and carbon-rich exudates, and more. In fact, between 5 and 33 percent of fixed atmospheric carbon is sequestered in the rhizosphere. The microbial communities that inhabit this niche can have a net beneficial or net detrimental impact on plant health, and shifting this balance is of major agronomic interest. Various mutualistic rhizosphere microbes provide the host plant with physiologically accessible nutrients; improve plant growth through production of phytohormones; help plants withstand heat, salt, and drought; act as protectants against phytopathogens; and more.

Microbial community structure differs across plant species and also among some inbred genotypes within single species grown in a common soil. Studies abound in a variety of systems to address the host genetic effect on the microbiome for various crops and other plants. Rhizosphere and microbial community analysis studies in *Arabidopsis* are also becoming more common, although these lack power because they use methods that are not readily comparable among studies, use low-resolution phylotyping techniques, and suffer from small sample sizes.

To define a robust system in which host genes important in shaping definable microbial phenotypes could be subsequently identified. Such host genes would constitute practical targets for intervention in crop plants to promote plant health in particular soil and climate conditions. One critical question is whether the gain and loss of effectors, and their subsequent recognition, play a role in the evolution or interconversion of pathogens and mutualists. Another is to what degree the known components of the plant immune system modulate the assemblage of the root microbiome. These new projects will benefit from integration with our ongoing studies of both effector diversity and plant immune function.

Using *Arabidopsis thaliana* inbreds and related Brassicaceae, we applied multiplexed pyrosequencing of microbial 16S rRNA genes from the root systems of hundreds of individual plants to test the hypothesis that the microbiota of a plant grown in wild soils is sufficiently dependent on host genotype to vary among related inbred individuals. We found distinct microbial communities in bulk soil, rhizosphere, and endophyte fractions that are influenced by soil type and plant developmental stage. We also found that plant genotype directs the assembly of robust microbial phenotypes**,** setting the stage for genetic dissection of responsible host loci.

11. Bioconversion of Agricultural wastes

2.1. Solid-State Fermentation for Bioconversion of Agricultural and Food Processing Waste into Value-Added Products

Solid-state fermentation (SSF), a general method for food processing waste bioconversion, is a process in which microorganisms grow on or within solid substrates in the absence of free water. However, substrates must possess enough moisture to support the growth and metabolism of microorganisms (56). The solid material in this process acts both as physical support and source of nutrients. To simplify product isolation from the medium, for example, polyurethane foam may be used instead of natural raw material such as wheat bran. SSF has been conventionally more applicable for filamentous fungi, but yeast and even bacteria are successfully used for biotechnological production by solid-state fermentation. SSF is a low-level technology in comparison with industrial submerged fermentation, but it appears to be a promising technology for the utilization of solid wastes. SSF is of special interest to countries with an abundance of agro-industrial residues that can be used as inexpensive raw materials. SSF has many advantages in processing agro-industrial residues as compared with submerged fermentation: lower energy requirements, process simplicity, cheaper aeration, absence of rigorous control of fermentation parameters and production of smaller quantity of

Bioconversion of food processing waste in products

Waste	Value-added product	Microorganism
Apple pomace, cranberry pomace and strawberry pomace	Enzyme Polygalacturonase	*Lentinus edodes*
Wheat bran, sunflower flour, coffee husk, soybean meal, rice bran, corn bran, rice hull, aspen wood, sweet potato residue, waste hair	Enzymes Proteases (acidic, neutral and alkaline)	*Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Bacillus* sp., *Trichoderma* sp.
The mixture of sugar cane bagasse with wheat bran or orange bagasse	Enzyme Pectin lyase and polygalacturonase	*Thermoascus aurantiacus*
Lemon pulp	Enzyme Pectinase	*Trichoderma viride*
Wheat bran, rice bran, apple pomace	Enzyme Pectinase	*Bacillus* sp.
Wheat bran, rice bran, coconut oil cake and corn flour	Enzyme Inulinase	*Staphylococcus* sp., *Kluyveromyces marxianus*
Lignocellulosic wastes, sugarcane bagasse	Enzymes Cellulase β-glucosidase	*Aspergillus ellipticus*, *Aspergillus fumigatus*
Banana waste	Enzymes α-amylase Cellulases	*Bacillus subtilis*
Tea waste	Enzyme Glucoamylase	*Aspergillus niger*

Value-Added Biotechnological Products from Organic Wastes

Table 8.3
(Continued)

Pineapple waste	Organic acid Citric acid	*Aspergillus foetidus* *Aspergillus niger*
Sugarcane bagasse	Amino acid L-glutamic acid	*Brevibacterium* sp.
Sweet potato residue	Antibiotic Tetracycline Chlorotetracycline	*S. viridifaciens*
Soybean curd residue, okara	Antibiotic Iturin A	*Bacillus subtilis*
Wheat bran	Plant growth hormone Gibberellic acid	*Gibberella fujikuroi*
Cassava flour, sugar cane bagasse	Plant growth hormone Gibberellic acid	*Gibberella fujikuroi*
Hydrolyzed tomato pomace	Vitamin B_{12}	*Propionibacterium shermanii*
Prawn-shell waste	Single cell protein	*Candida* spp. *Rhodotorula* spp.
Olive pomace after delignification and saccharification	Poultry feed enriched by protein	*Candida utilis* or *Saccharomyces cerevisiae*
Apple pomace	Animal feed	*Aspergillus niger* and

Hydrolyzed potato starch waste	Exopolysaccharide Pullulan	*Aureobasidium pullulans*
Grape skin pulp extract, starch waste, olive oil waste effluents, molasses	Exopolysaccharide Pullulan	*Aureobasidium pullulans*
Sent malt grains, apple pomace, grape pomace, and citrus peels	Exopolysaccharide Xanthan	*Xanthomonas campestris*
Olive mill wastewaters	Exopolysaccharide Xanthan	*Xanthomonas campestris*
Coconut waste	Bacterial endotoxins	*Bacillus thuringiensis*

POSSIBLE QUESTIONS
UNIT-I
PART-A (20 MARKS)
(Q.NO 1 TO 20 Online Examination)

PART-B (2 MARKS)

1. Comment on biological nitrogen fixation
2. What is symbiosis
3. What is role of *Nif* gene
4. What is rhizosphere and rhizoplane
5. Comment on the importance of beneficial microbes for crop improvements

PART-C (8 MARKS)

1. Describe the genetically modified organisms
2. Explain the role of symbiotic microbes to biological nitrogen fixation
3. Give a detailed notes about the microbial conversion of agricultural wastes
4. Comment on the biochemistry of nitrogen fixation
5. Describe the microbial interaction with plants

S.No	Unit IV	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	_____ are aerobic and free-living nitrogen nitrogen fixers	Frankia & Azospirillum	*Clostridium& Desulfovibrio*	Beijerinckia & Klebsiella	*Rhizobium& Anabaena*	Beijerinckia & Klebsiella
2	_____ are genes encoding enzymes involved in the fixation of atmospheric nitrogen	*mif*	*nif*	*sif*	*nod*	*nif*
3	_____ catalyze conversion of atmospheric nitrogen to ammonia	Kinase	Hydrogenase	Nitrogenase	Phosphatase	Nitrogenase
4	_____ is a typical example of symbiotic nitrogen fixation seen in paddy fields	Azolla-Anabaena	Alder-Frankia	Legume-Rhizobium	Higher plants-Mycorrhizae	Azolla-Anabaena
5	_____ recycles the H2 produced during N2 fixation, thereby minimizing the loss of energy	Reductase	Catalase	Nitrogenase	Hydrogenase	Hydrogenase
6	A free-living anaerobic photosynthetic bacterium	*Anabaena azollae*	*Clostridium thermocellum*	*Rhodospirillum rubrum*	*Klebsiella pneumoniae*	*Rhodospirillum rubrum*
7	A free-living soil bacteria that is involved in nitrogen fixation	*Alcaligenes*	*Acetobacter*	*Pseudomonas*	*Azotobacter*	*Azotobacter*
8	Amount of ATP needed to form 2 moles of ammonia from 1 mole of nitrogen gas during biological nitrogen fixation	8	16	32	64	16
9	Apart from biological nitrogen fixation by microbes,_____can fix atmospheric nitrogen	Cyclone	Thunder	Raining	Lightning	Lightning
10	Bacteria that forms root nodules in legume plants	*Rhizobium*	*Azotobacter*	*Azospirillum*	Cyanobacteria	*Rhizobium*
11	Biological nitrogen fixation was discovered by	Winogradsky	Beijerinck	Pasteur	Koch	Beijerinck
12	Chemicals produced by the Rhizobia called_____that cause the colonized root hairs to curl	Pod factors	Nod factors	Sod factors	Mod factors	Nod factors
13	Example of associative nitrogen fixation	Legume-Rhizobium	Rice-Azospirillum	Higher plants-Mycorrhizae	Azolla-Anabaena	Rice-Azospirillum
14	*Frankia is a*	Bacteria	Actinomycete	Fungi	Algae	Actinomycete

15	Group of irregularly shaped bacteria in root nodules are called as	Bacteroids	Asteroids	Mesteroids	Histeroids	Bacteroids
16	In biological nitrogen fixation, ____ moles of ammonia are produced from one mole of nitrogen gas	2	4	6	8	2
17	In Cyanobacteria, nitrogen fixation occurs in terminally differentiated cells known as	Cyanocysts	Nitrocycts	Heterocysts	Homocysts	Heterocysts
18	In root nodules,_____bind and regulate the levels of oxygen in the nodule	Teghemoglobin	Peghemoglobin	Leghemoglobin	Hemoglobin	Leghemoglobin
19	Legume plants belongs to	*Solanaceae*	*Rosaceae*	*Astraceae*	*Fabaceae*	*Fabaceae*
20	Most abundant gas in atmosphere	Nitrogen	Oxygen	Carbon dioxide	Hydrogen	Nitrogen
21	Nitrogenase enzyme consists of	Iron protein	Molybdenum-iron protein	Iron protein and a molybdenum-iron protein	Hemoglobin	Iron protein and a molybdenum-iron protein
22	Rhizobia are attracted to _____ released by the host legume's roots	Flavonoids	Enzymes	Toxins	Chemicals	Flavonoids
23	The enzyme nitrogenase is inhibited by	CO2	Sulfur	Hydrogen	Oxygen	Oxygen
24	*Which is not true about Anabaena and Nostoc*	Filamentous	Nitrogen fixing	Cyanobacteria	Symbiotic	Symbiotic
25	The majority of hydrogenases in prokaryotes are_____containing enzymes	Nickel	Copper	Molybdenum	Sulfur	Nickel
26	The majority of hydrogenases in prokaryotes are_____containing enzymes	Nickel	Copper	Molybdenum	Sulfur	Nickel
27	With associative nitrogen fixation, which one of the following genera is associated?	Azotobacter	Escherichia	Rhizobium	Anabena	Azotobacter
28	The conversion of nitrogen to ammonia or nitrogenous compounds is called as_____	Nitrogen assimilation	Nitrogen fixation	Denitrification	Nitrification	Nitrogen fixation
29	Symbiotic nitrogen cyanobacteria are present in all except_____	Anthoceros	Azolla	Cycas	Gnetum	Gentum

30	All the following are free living nitrogen fixers except_____	Rhizobium	Azotobacter	Rhodospirillum	Clostridium	Rhizobium
31	Anabena is a nitrogen fixer present in the root pockets of_____	Marselia	Salvinia	Pistia	Azolla	Azolla
32	Splitting of dinitrogen molecule into free nitrogen atom in biological nitrogen fixation is carried out by _____	hydrogenase	nitrogenase	dinitrogenase	nitrate reductase	nitrogenase
33	Which of the following aid plants in the acquisition of nitrogen from nitrogen gas of the atmosphere?	Bacteria	Algae	Nematodes	Moulds	Bacteria
34	A major plant macronutrient found in nucleic acids and proteins is _____	calcium	nitrogen	sulphur	iron	nitrogen
35	Organisms capable of converting nitrogen to nitrate are_____	yeast	bacteria	roundworms	moulds	bacteria
36	Conversion of nitrite to nitrate is carried out by _____	Nitrosomonas	Nitrosococcus	Nitrobacter	Clostridium	Nitrobacter
37	The nonsymbiotic bacteria which fix nitrogen live in the soil independently are	Azotobacter	Anabena	Rhizobium	Azolla	Azotobacter
38	Which of the following is not the biofertilisers producing bacteria?	Nostoc	Anabaena	Both (a) and (b)	Clostridium	Clostridium
39	Which of the following is capable of oxidizing sulfur to sulfates?	*Thiobacillus thiooxidans*	*Desulfotomaculum*	*Rhodospirillum*	*Rhodomicrobium*	Thiobacillus thiooxidans
40	Nitrifying bacteria can not be isolated directly by the usual techniques employed to isolate hetrotrophic bacteria. The reasons may be due to	slow growth	medium growth	fast growth	no growth	slow growth
41	_____ play a key role in the transformatio nof rock in the transformation of rock to soil	Cyanobacterium	pectin decomposing bacteria	denitrifying bacteria		Cyanobacteria
42	Denitrification may be distinguished as	dissimilative and assimilative	assimilative	Partially dissimilative	Partially assimilative	Dissimilative and assimilative
43	All of the following are examples of negative symbiosis_____	amensalism	competition	commensalism	parasitism	competition
44	The reservior for nitrogen is _____	the atmosphere	rocks	ammonia	nitrates	the atmosphere

45	Most soil protozoa are flagellates or amoebas, having their dominant mode of nitrogan as	Ingestion of bacteria	ingestion of mold	ingestion of fungi	ingestion virus	ingestion of bacteria
46		slow growth	medium growth	fast growth	Good growth	slow growth
47	Nitrifying bacteria can not be isolated directly by the usual techniques employed to isolate hetrotrophic bacteria. The reasons may be due to					
48	The transformation of nitrates to gaseous nitrogen is accomplished by microorganisms in a series of biochemical reactions. The process is known as _____	nitrification	denitrification	nitrogan fixation	ammonification	Denitrification
49	Nitrogen fixation refers to the direct conversion of atmospheric nitrogen gas into_____	ammonia	glucose	ATP	Nitrate	ammonia
50	The diagnostic enzyme for denitrification is_____	nitrate reductase	nitrate oxidase	nitro oxidoreductase	reductase	nitrate reductase
51	An example of a symbiotic nitrogen fixer is_____	Azotobacter	Beijerinckia	Clostridium	Rhizobium	Rhizobium
52	In the process of nitrogen fixation, which of the following microorganism is involved?	Non symbiotic microorganisms only	Symbiotic microorganisms only	Non symbiotic and symbiotic microorganisms only	Symbiosis	Non symbiotic and symbiotic microorganisms only
53	The physical structure of soil is improved by the accumulation of _____	mold mycelium	minerals	water	metals	Mold mycelium
54	_____ play a key role in the transformation of rock to soil	Cyanobacteria	Pectin decomposing bacteria	Nitrifying bacteria	De-nitrifying bacteria	Cyanobacteria
55	The conversion of molecular nitrogen into ammonia is known as_____	nitrification	denitrification	nitrogen fixation	ammonification	nitrogen fixation
56	Some microorganisms have the ability to increase the nitrogen content of soils, are called as	Nitrogen fixation	denitrification	nitrification	ammonification	nitrogen fixation

57	Which of the following soil microorganism is involved in the reduction of sulfates to H2S	Thiobacillus thiooxidans	*Desulfotomaculum*	*Rhodospirillum*	*Rhodomicrobium*	*Desulfotomaculum*
58	Which of the following fungi on infecting crop roots can improve their uptake of phosphorus and other nutrients?	*Saccharomyces cerevisiae*	VA Mycorrhiza	*Candida torulopsis*	*Aspergillus niger*	VA Mycorrhiza
59	Syntrophism involves	exchange of nutrients between two species	exchange of nutrients among species	no exchange of nutrients between two species	no exchange of nutrients among species	exchange of nutrients between two species
60	Assimilative denitrification is done by	Plants	animals	virus	protozoans	plants
61	The diagnostic enzyme for nitrogen-fixing organisms is	nitrogenase	nitrate reductase	nitrate oxidase	dehrogenase	nitrogenase
62	The groups of bacteria which have the ability to fix nitrogen from air to soil are	Symbiotic	Antagonostic	Mutualistic	synergistics	Symbiotic
63	The nitrogenase consists of	dinitrogenase	reductase	hydrogenase	nitrogenase	dinitrogenase
64	The conversion of molecular nitrogen into ammonia is known as	nitrification	denitrification	nitrogen fixation	ammonification	nitrogen fixation
65	Some microorganisms have the ability to increase the nitrogen content of soils, are called as	nitrogen fixation	denitrification	nitrification	nitrogenase	nitrogen fixation
66	Which are the main source of biofertilisers?	Cyanobacteria	Bacillus	Streptococcus	Azolla	Cyanobacteria
67						
68	The physical structure of soil is improved by the accumulation of _____	Mold mycelium	minerals	water	yeast	mold mycelium
69	_____ play a key role in the transformatio nof rock in the transformation of rock to soil	Cyanobacterium	pectin decomposing bacteria	denitrifying bacteria		Cyanobacteria
70	Denitrification may be distinguished as	dissimilative and assimilative	assimilative	Partially dissimilative	Partially assimilative	Dissimilative and assimilative
71	All of the following are examples of	amensalism	competition	commensalism	parasitism	competition

	negative symbiosis_____					
72	The reservior for nitrogen is _____	the atmosphere	rocks	ammonia	nitrates	the atmosphere
73	When a host associates with other organism for either benefit or completion of their life cycle is known as _____	Cooperation	Mutualism	Symbiosis	Synergism	Symbiosis
74	An obligatory relationship between host and its symbionts is known as	Cooperation	Mutualism	Symbiosis	Synergism	Mutualism
75	Which onr of the following presence and contributions of microorganisms through their activities to the places where they are found	Environmental microbiology	Microbial ecology	Microbial physiology	Microbial taxonomy	Microbial ecology
76	The relationship in which one symbionts benefits while the other is neither harmed nor help is known as	Cooperation	Synergism	Syntrophism	Commensalism	Commensalism
77	Which of the following describes that one organism has deverse effect on another organism	amensalism	Commensalism	Mutulism	Cooperation	Amensalism
78	Which of the following describes an example of ammensalism in soil ecosystem	Production of antibiotics	Production of secondary metabolite	Production of geosmine	Production of primary metabolites	Production of antibiotics
79	The aphids is an excellent example of which one of the following relationship	Mutualism	Cooperation	Symbiosis	Synergism	Mutualism

Application of biofertilizers and biomanures – A combination of biofertilizer and manure applications with reference to soil, seed and leaf sprays. Plant growth promoting microorganisms- Myzorrhizae, Rhizobia, Azosprillum, Azotobacter, Azolla, Frunkia, Blue green algae, Phosphate- solubilizers fluorescent *Pseudomonas*. Laboratory and field application; Cost-benefit analysis of biofertilizer and biomanure production. Biocontrol and its application: Biofungicides, bionematicides and Biopesticides.

APPLICATION OF BIOFERTILIZER AND BIOMANURE

Materials of biological origin commonly used to maintain and improve soil fertility are called bio-fertilizers. These are categorized as- Manures and Bio-fertilizers

MANURES

Manures are organic wastes that after partial decay are added to the soil to increase crop productivity.

GREEN MANURE

Manures supply all essential elements required by the crop plants.
They Improve physical condition of soil.
They also add to water holding capacity of soil.
KINDS OF MANURE

GREENMANURE

Quick growing leguminous crops are grown and ploughed when they are about one foot in height.
This supplies the soil with organic as well as inorganic components to the soil.It also provides a protective action against erosion and leaching.Crops like cluster beans (Cyamopsis tetragonoloba), horse gram (Macrotyloma uniforma), lentil or masur (Lens esculenta) etc., are grown as green manure crops.

FARMYARDMANURE

It is obtained by partial decay of animal dung, farm refuse and crop residues.
Manure consists of colloidal particles that help to imbibe water and loosen the soil, increase its aeration and permit easy penetration of roots in the soil. It enriches the soil with many organic substances and releases mineral element in the soil.

Residue of gobar gas plant is a kind of farmyard manure.

COMPOSTMANURE

It consists of rotten vegetables, animal refuse, groundnut husk and other substances.

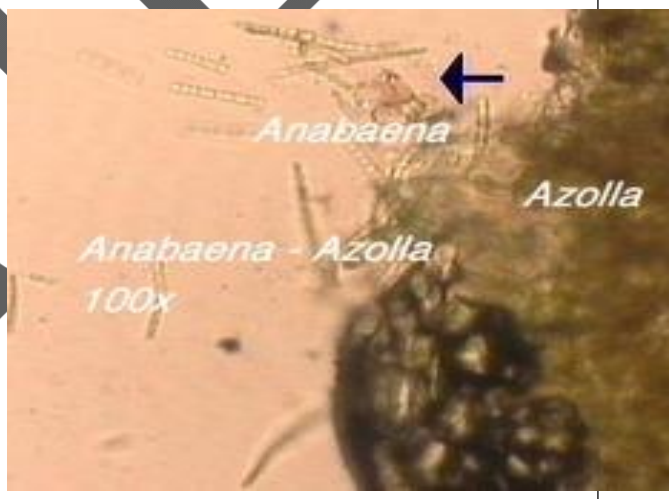
It is prepared by dumping these substances in heaps with sprinkling of chemical fertilizers like ammonium sulphate, superphosphate etc. It takes about 4-6 months to form compost manure.

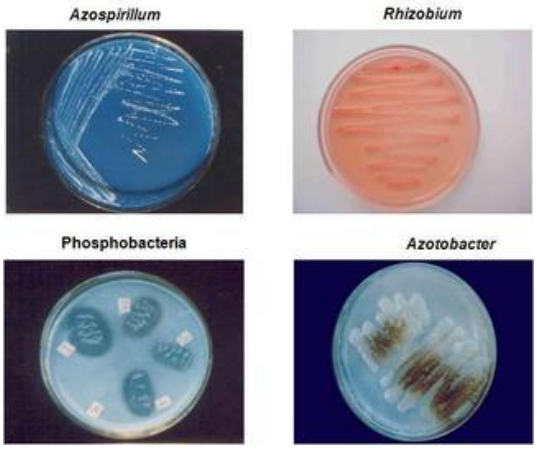
BIOFERTILIZERS

Microorganisms that enrich the soil in nutrients by their biological activity are bio-fertilizers. Main sources are- bacteria, cyanobacteria and fungi.

FARMYARD MANURE

COMPOSTMANURE





Bio-fertilizers: Types and Importance of Bio-fertilizers!

Chemical fertilizers are being used in increasing amounts in order to increase output in high yielding varieties of crop plants.

However, chemical fertilizers cause pollution of water bodies as well as ground water, besides getting stored in crop plants.Therefore, environmentalists are pressing for switch over to organic farming.Organic farming is the raising of unpolluted crops through the use of manures, bifertilizers and biopesticides that provide optimum nutrients to crop plants, keeping pests and pathogens under control.

Bio-fertilizers are micro-organisms which bring about nutrient enrichment of soil by enhancing the availability of nutrients to crops. The micro-organisms which act as bio-fertilizers are bacteria, cyanobacteria (blue green algae) and mycorrhizal fungi. Bacteria and cynobacteria have the property of nitrogen fixation while mycorrhizal fungi preferentially withdraw minerals from organic matter for the plant with which they are associated.

Nitrogen fixation is the process of conversion of molecular or dinitrogen into nitrogen compounds. Insoluble forms of soil phosphorus are converted into soluble forms by certain micro-organisms. This makes the phosphorus available to the plants. Phosphate is also solubilised by some bacteria and by some fungi that form association with plant roots.

The various bio-fertilizers are as follows.

(i) Free Living Nitrogen Fixing Bacteria:

They live freely in the soil and perform nitrogen fixation. Some of them are saprotrophic, living on organic remains, e.g., Azoto- bacter, Bacillus polymyxa, Clostridium, Beijerinckia. They are further distinguished into aerobic and anaerobic forms.

The property of nitrogen fixation is also found in photoautotrophic bacteria, e.g., Rhodopseudomonas, Rhodospirillum, Chromatium. Inoculation of soil with these bacteria helps in increasing yield and saving of nitrogen fertilizers. For example, Azotobacter occurring in fields of Cotton, Maize, Jowar and Rice, not only increases yield but also saves nitrogen fertilizer to the tune of 10-25 kg/ha. Its inoculation is available under the trade name of azotobactrin.

(ii) Free Living Nitrogen Fixing Cyanobacteria:

A number of free living cyanobacteria or blue-green algae have the property of nitrogen fixation, e.g., Anabaena, Nostoc, Aulosira, Totypothrix, Cylindrospermum, Stigonema. Cyanobacteria are photosynthetic. Therefore, they add organic matter as well as extra nitrogen to the soil.

Aulosira fertilissima is considered to be the most active nitrogen fixer of Rice fields in India (Aiyer et al, 1972). Cylindrospermum licheniforme grows in Sugarcane and Maize fields. Cyanobacteria are an extremely low cost biofertilisers. In Tamil Nadu, the technique of cyanobacteria inoculation to rice fields is being followed. Phosphate, Molybdenum and Potassium are supplied additionally.

(iii) Loose Association of Nitrogen Fixing Bacteria:

Certain nitrogen fixing bacteria like Azospirillum live around the roots of higher plants without developing any intimate relationship. It is often called rhizosphere association. The bacteria obtain some plant exudate and use the same as part of their food requirement. The bacteria fix nitrogen and exude a part of the fixed nitrogen for use by the plant. The phenomenon is termed as associative mutualism (= associative symbiosis).

(iv) Symbiotic Nitrogen Fixing Bacteria:

They form a mutually beneficial association with the plants. The bacteria obtain food and shelter from plants. In return, they give a part of their fixed nitrogen to the plants. The most important of the symbiotic nitrogen fixing bacteria is Rhizobium (pi Rhizobia). It forms nodules on the roots of legume plants. There are about a dozen species of Rhizobium which form association with different legume roots, e.g., R. leguminosarum, R. lupini, R. trifolii, R. meliloti, R. phaseoli.

These bacteria, also called rhizobia, live freely in the soil but cannot fix nitrogen except for a strain of Cowpea Rhizobium (Me Comb et al, 1975). They develop the ability to fix nitrogen only when they are present inside the root nodules. In the nodule cells, bacteria (bacteroids) lie in groups surrounded by membrane of the host which is lined by a pink-red pigment called leghaemoglobin. Presently cultures of Rhizobium specific for different crops are raised in the laboratory.

Frankia, a nitrogen fixing mycelial bacterium (actinomycete), is associated symbiotically with the root nodules of several nonlegume plants like Casuarina, Alnus (Alder) Myrica, Rubus etc. Leaves of a few plants (e.g., Ardisia) develop special internal cavities for providing space to symbiotic nitrogen fixing

bacteria, Xanthomonas and Mycobacterium. Such leaves are a constant source of nitrogen fertilizer to the soil.

(v) Symbiotic Nitrogen Fixing Cyanobacteria:

Nitrogen fixing cyanobacteria (blue- green algae) form symbiotic association with several plants, e.g., cycad roots, lichens, liverworts, Azolla (fern). Out of these, Azolla-Anabaena association is of great importance to agriculture.

Azolla pinnata is a small free floating fresh water fern which multiplies rapidly, doubling every 5-7 days. The fern can coexist with rice plants because it does not interfere with their growth. In some South-East Asian countries, especially China, the rice fields are regularly provided with Azolla.

Anabaena azollae resides in the leaf cavities of the fern. It fixes nitrogen. A part of the fixed nitrogen is excreted in the cavities and becomes available to the fern. The decaying fern plants release the same for utilization of the rice plants. When field is dried at the time of harvesting, the fern functions as the green manure, decomposing and enriching the field for the next crop.

(vi) Microphos Biofertilizers:

They release phosphate from bound and insoluble states, e.g., Bacillus polymyxa, Pseudomonas striata, Aspergillus species.

vii) Mycorrhiza (pl-Mycorrhizae Frank,. 1885):

It is a mutually beneficial or symbiotic association of a fungus with the root of a higher plant. The most common fungal partners of mycorrhiza are Glomus species. Mycorrhizal roots show a sparse or dense wooly growth of fungal hyphae on their surface. Root cap and root hairs are absent.The shape is irregular, tuberous, nodulated or coralloid. The fungus remains restricted to the cortex of the root. The vascular strand and growing point are not affected. Mycorrhiza often remains in the upper layers of the soil where organic matter is abundant. Depending upon the residence of the fungus, mycorrhizae are of two types— ectomycorrhiza and endomycorrhiza.

(a) Ectomycorrhiza (= Ectotrophic Mycorrhiza):

The fungus forms a mantle on the surface of the root. Internally, it lies in the intercellular spaces of the cortex. The root cells secrete sugars and other food ingredients into the intercellular spaces for feeding the fungal hyphae. The exposed fungal hyphae increase the surface of the root to several times. They perform several functions for the plant— (i) Absorption of water,(ii) Solubilisation of organic matter of the soil humus, release of inorganic nutrients, absorption and their transfer to root,(iii) Direct absorption of minerals from the soil over a large area and handing over the same to the root. Plants with ectomycorrhiza are known to absorb 2-3 times more of nitrogen, phosphorus, potassium and calcium,(iv) The fungus secretes antimicrobial substances which protect the young roots from attack of

pathogens. Ectomycorrhiza occurs in the trees like Eucalyptus, Oak (Quercus), Peach, Pine, etc. The fungus partner is generally specific. It belongs to basidiomycetes.

(b) Endomycorrhiza (- Endotrophic Mycorrhiza):

Fewer fungal hyphae lie on the surface. The remaining live in the cortex of the root, mostly in the intercellular spaces with some hyphal tips passing inside the cortical cells, e.g., grasses, crop plants, orchids and some woody plants. In seedling stage of orchids, the fungal hyphae also provide nourishment by forming nutrients rich cells called pelotons. Intracellular growth occurs in order to obtain nourishment because unlike ectomycorrhiza, the cortical cells do not secrete sugars in the intercellular spaces.The hyphal tips passing into cortical cells either produce swollen vesicles or finely branched masses called arbuscules. Therefore, endomycorrhiza is also called VAM or vesicular-arbuscular mycorrhiza. The major benefits of VAM to the plant are the supply of inorganic nutrients as well as enhanced water absorption. Phosphate which is mostly present in the unavailable form in the soil, becomes abundantly available to the plant. A single fungus may form mycorrhizal association with a number of plants, e.g., Glomus.

Importance of Bio-fertilizers:

(i) They increase the yield of plants by 15-35%.(ii) Bio-fertilizers are effective even under semi-arid conditions,(iii) Farmers can prepare the inoculum themselves,(iv) They improve soil texture,(v) Bio-fertilizers do not allow pathogens to flourish,(vi) They produce vitamins and growth promoting bio-chemical's,(vii) They are non-polluting.

COMBINATION OF BIOFERTILOIZER AND MANURE WITH REFERENCE OF SOIL, ROOT AND LEAF

he rising

concerns on the risk of food safety, environmental pollution,and pathogen resistance as well as increasing policy demandfor reduction of chemical fertilizers and biocides require alter-natives in nutrient application and disease management.Organic fertilizers have been employed to replace chemicalfertilizers in many areas for soil remediation. Fertilizers fromplant and fungal resources are considered safer than thosefrom animal resources since heavy metals and antibioticshave been detected in the latter (Nicholson et al., 1999; Martınez-Carballo et al., 2007). Spent mushroom substrate (SMS),the unutilized substrate and the mushroom mycelium left afterharvest of mushrooms, is an organic by-product generated inthe mushroom industry. The increasing volume of SMS alongwith the growing industry has raised people's concerns on itstreatment and environmental impact.he risingconcerns on the risk of food safety, environmental pollution,and pathogen resistance as well as increasing policy demandfor reduction of chemical fertilizers and biocides require alter-natives in nutrient application and disease management.Organic fertilizers have been employed to replace chemicalfertilizers in many areas for soil remediation. Fertilizers fromplant and fungal resources are considered safer than thosefrom animal resources since heavy metals and antibioticshave been detected in the latter (Nicholson et al., 1999; Martınez-Carballo et al., 2007). Spent mushroom substrate

(SMS),the unutilized substrate and the mushroom mycelium left afterharvest of mushrooms, is an organic by-product generated inthe mushroom industry. The increasing volume of SMS alongwith the growing industry has raised people's concerns on itstreatment and environmental impact.

Bio-fertilizer and organic manure are cheap and eco-friendly source of plant nutrients for sustainable crop production in low-input agriculture. The role of biofertilizers alone or in combination with organic or inorganic fertilizers has recently gained recognition in sustainable crop production (Kennedy et al., 2004; Bloemberg et al., 2000; Abdullahi and Sheriff, 2013). These microorganisms play crucial roles such as, producing plant growth stimulating hormones, nutrients cycling thereby enhancing plant nutrients availability and uptake, nitrogen fixation, improve plant health, drought resistance and, reclaim degraded soil (Barea et al., 1998; Dobbelaere et al., 2001; Hodge et al., 2001; Bonfante, 20003; Vassey, 2003). Application of organic manures similarly, has positive effects on soil physical and biochemical properties. It lowers soil bulk density; increases water holding capacity, CEC, build up beneficial soil microbes, improve good soil structure and enhance stable soil aggregates (Doran, 1995; Drinkwater et al., 1995; Stamatiadis et al., 1999). The objective of this work was to study the effect of bio-fertilizer, Arbuscular mycorrhizal fungi (AMF) (Glomus mossea) and Azospirillum brasilense alone or in combination with cow dung (CD) or poultry manure (PM) on growth of pearl millet.

Plant growths characteristics viz; plant height, number of tillers/plant, shoot and root dry biomass are presented in Figure-1 and Table-1. Bio-fertilizers and organic manures either alone or in combination significantly produced plants with high growth qualities compared to control Combination of bio-fertilizer with poultry manure (Bio- fertilizer + PM) produced plants with the highest growth biometrics; plant height (72.6 cm), number of tillers/plant (4.1), shoots and root dry biomass (8.8 and 3.9 g) followed by single applications of biofertilizer and poultry manure. Applying 10 ton-1 of cow dung produced plants with the lowest growth attributes although not a par with control.

application of biofertilizer and organic manure alone or in combination enhanced plant growth, % root colonization by AM fungi, shoot and root dry biomass and nutrients concentration (N, P and K) compared to control. Bio-fertilizer + PM recorded highest plant performance followed by biofertilizer alone. Improved plant growth could be attributed to the energy source provided to the microbes via organic manure thereby enhancing biological activities and availability of nitrogen, growth promoting hormones and phosphorus mobilization by Azospirillum and AM fungi, respectively. Several studies have revealed the positive effects of organic and bio-fertilizers singly or in combination with organic amendments to increase plant nutrients availability, uptakes and increase crop yield (Ezhil Bama and Ramakrishnan, 2010; Saxena and Tilak, 1994; Nadar et al., 2008; Maman and Mason, 2013). Similar to the finding of this study, Abdullahi et al. (2013) reported enhanced sesame growth and nutrients uptake with the combination of bio-fertilizer and poultry manure. Osman and Abd El-Rahman (2010) observed high growth response, quality yield of fruit and leaf nutrients concentrations (N, P, K, Ca and Mg) in Fig with the application of Azospirillum + poultry manure and Azotobacter + poultry manure. Positive growth response of pearl millet to bio-fertilizer application compared to chemical fertilizers was also reported by Campo (2006) and Galbiatti et al. (2011). Root colonization recorded in un-inoculated plants

in this study might be due to presence of natural AM fungi spores in the experimental plots. Since present study was conducted under natural field conditions where, native AM fungi spores could be present, with low population and poor colonization potential. Combined effect of bio-fertilizer + PM and single application of PM were superior to bio-fertilizer + CD and CD alone; this is due to differences in chemical composition and wider C/N ratio of the cow dung. Contrary to the result of this study, Sadiq et al. (2012) recorded highest growth and yield of pearl millet treated with cow dung compared to poultry manure under the same agro-ecological condition where this study was conducted. From the findings of this study, it can be concluded that application of bio-fertilizer and organic manure either singly or in combination could improve pearl millet production in low-input agriculture. Results also suggested that bio-fertilizer have reduced by half the application rates of organic manure. Bio-fertilizer in combination with 2.5 ton ha-1 of PM could be recommended for millet production in the study area.(ARPN Journal of Agricultural and Biological Science, VOL. 9, NO. 10, OCTOBER 2014)

Constant use of land leads to loss of its fertility and thus the fertility needs to be replenished.The deficiency of any one or more nutrients in the soil may impair the growth and development of plants. Macroelements like nitrogen, phosphorous and potassium are required in larger quantities. These are referred to as the NPK fertilizers.
CHEMICAL fertilizers like nitrogen, and phosphorous are applied to the land so that it regains its fertility.

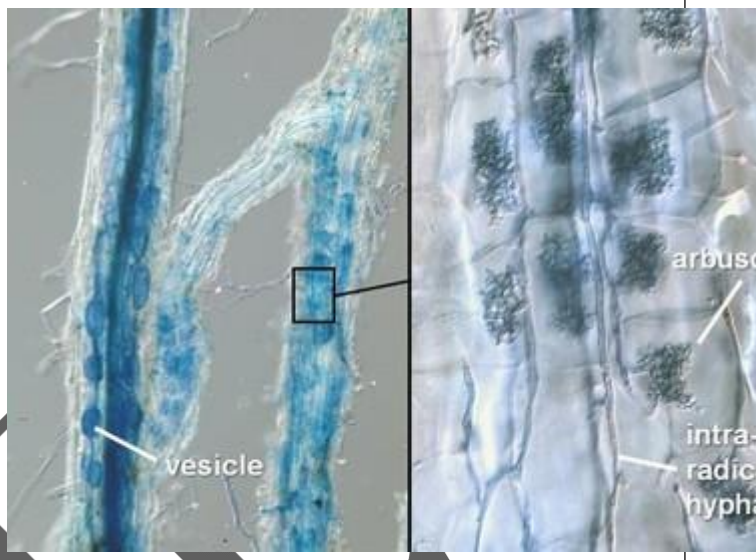
FUNGI AS BIOFERTILIZERS

MYCORRHIZA

Symbiotic relationship between fungal hyphae and roots of higher plants is known as mycorrhiza.

The fungi obtain food from the plant and gives mineral elements in return. Mycorrhizal association converts a marginal land into a fertile land and reduces dependency on irrigation and fertilizers.

According to their relationship they are classified as ectomicorrhiza and endomicorrhiza



ECTOMICORRIZA

Fungal hyphae form a dense sheath external to the root.

They are found on the roots of forest trees like Pine, Oak etc.

They absorb Nitrogen, Phosphorous potassium and calcium, they also convert complex organic molecules into simpler available forms.

ENDOMYCORRHIZA

Endomycorrhiza in association with cortex cells of roots.

Some penetrate the roots and form vesicles and arbuscules in t cortex.

This type of fungi are called vescicular-arbuscular micorrhizal (V fungi.

Eg. Acaulospora, glomus, gigaspora etc.

PESTICIDES

Pests are agents that damages the economic and physical well being of human beings.

Pesticides are chemicals which kill or repel the pests.

ADVANTAGESANDDISADVANTAGES

Advantage-

Itisusedtoincreasetheyield.

It is also used to control vector borne diseases.

Disadvantages-
It is injurious to man and animals.
It causes destruction of ecosystem
Pesticides also destroy other organisms along with the target organisms.
The pests that survive become pest resistant and further breed pest resistant progeny.
It causes pollution.

Pesticides like organophosphates are stable compounds and cannot be broken down easily. Thus they tend to get accumulated in the body of the animals. This increase in the pesticides in the body of the organisms is called bio-magnification.

BIOLOGICAL METHOD OF PEST CONTROL

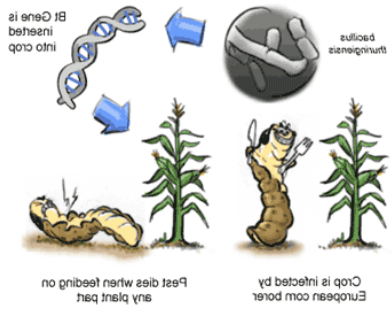
BIOHERBICIDES

Weeds are undesirable plants that grow along with the crops and compete with them for food, space and water.
The modern technique of biological control of weeds, that involve the use of insects and microorganisms to feed on these weeds, and not on the crops, are called bioherbicides.
The insects used as bioherbicides are first tested with regard to their specificity on a particular weed.
Examples of weed control by bio herbicides.
1) The first effective bioherbicide was a mycoherbicide phytophthora plamivora, which controls the growth of milk weed units in citrus orchards.
2) Growth of cacti in India was checked by the introduction of the natural herbivore cochineal insect cactoblastis cactorum.
3) Attempts to control water hyacinth in India were made by the application of Alternaria eichhorniae.
4) Plant breeders have developed transgenic tomato and tobacco plants by genetic engineering by introducing the genes for herbicidal resistance into crop plants so that the new plants became herbicidal resistant. The herbicide kills the weeds selectively and the transgenic crop plants remain healthy.

Cochinealinsect-
Cactoblastis cactorum was first introduced to Australia in 1925 from Argentina, where it was successfully utilized as a biological control agent for Opuntia cacti. Due to this success, it was subsequently introduced into other countries. A primarily sessile parasite, this insect lives on cacti from the genus Opuntia, feeding on plant moisture and nutrients. The insect produces carminic acid that deters predation by other insects.

BIO-INSECTICIDES

Bio-insecticides are organic formulations recommended for the management of insects that feed on crops. They



are different from chemical pesticides in several ways. They contain live bacteria that produce toxins which cause stomach poison in the insects and kill them.

Bacillus thuringiensis (or Bt) is a Gram-positive, soil-dwelling bacterium, commonly used as a pesticide. B. thuringiensis also occurs naturally in the gut of caterpillars of various types of moths and butterflies, as well as on the dark surface of plants.
Bt has to be eaten to cause mortality. The Bt toxin dissolve in the high pH insect gut and become active. The toxins then attack the gut cells of the insect, punching holes in the lining. The Bt
spores spills out of the gut and germinate in the insect causing death within a couple days.
Even though the toxin does not kill the insect immediately, treated plant parts will not be damaged because the insect stops feeding within hours. Bt spores do not spread to other insects or cause disease outbreaks on their own.

HOW Bt WORKS.....

1. Insect eats Bt crystals and spores.
2. The toxin binds to specific receptors in the gut and the insects stops eating.
3. The crystals cause the gut wall to break down, allowing spores and normal gut bacteria to enter the body.
4. The insect dies as spores and gut bacteria proliferate in the body.

Bt action is very specific. Different strains of Bt are specific to different receptors in insect gut wall. Bt toxicity depends on recognizing receptors, damage to the gut by the toxin occurs upon binding to a receptor. Each insect species possesses different types of receptors that will match only certain toxin proteins, like a lock to a key.

It is the selection, integration and implementation of pest control based on economic, ecological and sociological consequences.
IPM is an "economically justified and sustainable system of protection of crops that leads to the maximum agricultural productivity with the least possible negative impacts on the natural environment".
The concept of Integrated Pest Management was developed by Dr. Ray Smith (1919 – 1999). He was an American Entomologist and educator, around 1950. The Integrated Pest Management Programme (IPM) is now a worldwide programme which lays emphasis on the application of Bio-pesticides, and Bio-agents with rarest and unavoidable application of safe chemical pesticides.
It involves following principles-

Cultural control- like crop rotation.

Mechanical control- like catching and killing the insects and rodents.

Genetic practise- Selection of comparatively pest resistant/tolerant varieties with reasonable yield levels

Chemical control- Use of chemical pesticides is the last resort when all other methods fail to keep the pest population below economic loss. Use of pesticides should be need based, judicious, based on pest surveillance and economic threshold level

Use of resistant varieties- like disease resistant wheat, rice etc.

Biological control- like use of parasites and predators to control pests.

Possible Questions:

Part A(2 Marks)

1. Discuss in detail the steps involved in the production of biofertilizer.
2. Comment on Azolla and green manure.
3. Give an account of bacterial insecticides.
4. Explain the importance of microbes in biomanure production.
5. Discuss about the Azosprillum inoculum.
6. Write notes on biofertilizer.

Part-C (8 Marks)

1. Give a brief note on cost benefit analysis of biofertilizer. Or
2. Give a detail note on Frunkia.
3. Detail in account of a combination of biofertilizer and manure applications with reference to soil,seed and leaf spray.
4. Describe the phosphate solubilizing organisms. Or
5. Give a detail note on significance of azolla in agriculture.
6. Discuss in detail about application of biofertilizer and biomanure. Or

S.No	Unit V	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	_____ is organic matter, mostly derived from animal waste/feces	Biomanure	Fertilizer	Potash	NPK	Biomanure
2	_____ is the used for seed treatment of groundnut	*Azospirillum*	*Azotobacter*	*Rhizobium*	*Nostoc*	*Rhizobium*
3	_____ are best phosphate mobilizers	*Mycorrhizae*	*Bacillus*	*Citrobacter*	*Candida*	*Mycorrhizae*
4	_____ is a biocontrol agent	*Bacillus polymyxa*	*Azospirillum*	*Trichoderma viridae*	*Aspergillus flavus*	*Trichoderma viridae*
5	_____ are rich in beneficial microorganisms that enrich the nutrient quality of soil	Biofertilizers	Humus	NPK	Vermicompost	Biofertilizers
6	_____ is a best biofertilizer used in paddy fields	*Bradyrhizobium*	*Azospirillum*	*Azolla*	*Frankia*	*Azospirillum*
7	_____ is a form of agriculture that relies on techniques such as crop rotation, green manure, compost, and biological pest control.	Terrestial farming	Hill farming	Inorganic farming	Organic farming	Organic farming
8	_____ is phosphate solubilizing bacteria	*Bacillus megaterium*	*Bacillus anthrax*	*Bacillus cereus*	*Bacillus phosphatae*	*Bacillus megaterium*
9	_____ is the biological oxidation of ammonia	Oxidation	Nitrification	Denitrification	Reduction	Nitrification
10	_____ can be used with crops like wheat, maize,mustard, cotton, potato and other vegetable crops	*Anabaena*	*Azotobacter*	*Rhizobium*	*Mycorrhizae*	*Azotobacter*
11	_____ is a plant growth promoting bacteria found naturally in soil	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Pseudomonas fluorescens*	*Aspergillus fumigatus*	*Pseudomonas fluorescens*

12	A carrier used in preparation of biofertilizers	Rubber	Peat	Plastic	Soil	Peat
13	A fertilizer consisting of growing plants that are ploughed back into the soil	Green manure	Vermicompost	Biomanure	Organic fertilizer	Green manure
14	Chemoautotrophic involved in nitrification	*Alcaligenes*	*Fusarium*	*Nitrosomonas*	*Arthrobacter*	*Nitrosomonas*
15	Cyanobacteria are	Photoheterotrophs	Chemotrophs	Prototrophs	Photoautotrophs	Photoautotrophs
16	Denitrification is a microbially facilitated process of	Nitrate degradation	Nitrate assimilation	Nitrate oxidation	Nitrate reduction	Nitrate reduction
17	Denitrifying bacteria	*Thiobacillusdenitrificans*	*Bacillus*	*Aspergillus*	*Micrococcus*	Thiobacillusdenitrificans
18	Enzyme involved in phosphate solubilization	Oxidases	Reductases	Kinases	Phytases	Phytases
19	Foliar spray is	Spraying on roots	Spraying on Stem	Spraying on leaves	Spraying on Flowers	Spraying on leaves
20	Indole acetic acid and gibberelins are	Hormones of bacteria	Hormones that retard plant growth	Plant growth hormones	Weedicides	Plant growth hormones
21	Liquid extract of composting by earthworms	Vermiwash	Germiwash	Wormiwash	Liquiwash	Vermiwash
22	Majority of atmospheric nitrogen is obtained from	Fossil fuel	Hospital waste	Sewage waste	Industrial waste	Fossil fuel
23	Microorganisms make soluble phosphate from insoluble phosphate by producing	Hydrochloric acid	Sulphuric acid	Nitric acid	Organic acids	Organic acids
24	PGPR is	Phosphorous growth promoting bacteria	Plant ibberellin promoting bacteria	Plant growth promoting biomass	Plant growth promoting bacteria	Plant growth promoting bacteria
25	Phyllosphere refers to	Surface of roots	Surface of leaves	Surface of Stem	Surface of flowers	Surface of leaves
26	Rhizobacteria are bacteria growing in & around_____of	Leaf	Root	Stem	Fruit	Root

	plants					
27	VAM is	Ventricular arbuscular mycorrhizae	Vesicular augumenting mycorrhizae	Vesicular arbuscular mycorrhizae	Vesicular arbuscular mycobacterium	Vesicular arbuscular mycorrhizae
28	Which are important nutrients for plant growth in soil?	Nitrogen	Phosphorous	NPK	Potassium	NPK
29	Which bacteria is used as biofertilizer in sugarcane crop?	*Beijerinckia*	*Acetobacter diazotrophicus*	*Bacillus*	*Pseudomonas*	*Acetobacter diazotrophicus*
30	Which forms symbiotic relation with higher plants?	*Aspergillus fumigatus*	*Bradyrhizobium*	*Pseudomonas fluorescens*	*Mycorrhizae*	*Mycorrhizae*
31	Expect Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants?	Actinorhiza	Burholderia	Micrococcus	Pseudomonas	Burholderia
32	Rhizobium has symbiotic association with_____	Legumes	non-legume crop	sugarcane	paddy	legumes
33	Which of the following is not the biofertilisers producing bacteria?	Nostoc	Anabena	Both a and	Clostridium	Clostridium
34	Which of the following is capable of oxidising sulfur to sulfates?	Thiobacillus thioxidans	Desulfotomaculum	Rhodospirilliu m	Rhodomicrobium	Thiobacillus thiooxidans
35	Azolla is used as biofertilizer as it has _____	Rizobium	Cyanobacteria	Mycorrhiza	Large quantity of humus	Cyanobacterium
36	The most quickly available surce of nitrogen to plants are_____	amide fertilizers	ammonia fertilizers	nitrate fertilizers	ammonia nitrate fertilizer	amide fertilizers
37	Most effective pesticide is _____	carbamates	organophosphates	organochlorine s	phosphates	carbamates

38	Which is true for DDT	not a pollutant	an antibiotic	an antiseptic agent	a non degradable pollutant	a non degradable pollutant
39	Which is major component of bordeaux mixture?	copper sulphate	sodium chloride	calcium chloride	magnesium sulphate	sodium chloride
40	Which one is correctly matched	carbamates-malathion	organophosphates -cabofuran	carbamates-malathion	organochloride-endosulphan	organochloride-endosulphan
41	IPM stands for	integrated plant manufacture	integrated plant management	integrated plant management	integrated pest management	integrated plant management
42	Which is major component of bordeaux mixture?	copper sulphate	sodium chloride	calcium chloride	magnesium sulphate	sodium chloride
43	Insecticides generally attack	respiratory system	muscular system	nervus system	circulatory system	muscular system
44	Organisms associated with sorghum and cotton which provide nutrition to them are	Azospirillum, Azotobacter	Azotobacter, Azospirillum	Anabena, Rhizobium	Rhizobium, Azotobacter	Azotobacter-Azospirillum
45	Azolla as biofertilizer, increase the yield of rice fields by	10%	20%	30%	50%	10%
46	Denitrification is _____	reduction of nitrate to nitrogen gas	reduction of nitrate to organic nitrogen compounds	both a and b	reduction of ammonia	Both a and b
47	Which of the following soil microorganism is involved in the reduction of sulfates to	Thiobacillus thiooxidans	Desulfotomaculum	Rhodospirilliu m	Rhodomicrobium	Desulfotomaculum

	hydrogen sulphide					
48	Which one of the following structure is formed in plant roots by mycorrhizae	Arbuscles	Hartig net	Haustoria	Rhizomorph	Hartig net
49	Except Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants_	Actinorhiza	Burholderia	Micrococcus	Pseudomonas	Burholderia
50	Which one of the following genes is responsible for nod factor in bacteria	fix gene	gag gene	nif gene	nol gene	nol gene
51	In which one of the following relationship one partner benefits but the other is neither hurt nor helpless	Amensalism	Commensalism	Parasitization	Predation	Commensalism
52	The proteinaceous compounds ae converted to ammonia in the presence of which one of the following bacteria	Ammonifying bacteria	Denitrifying bacteria	Nitrifying bacteria	Putrefying bacteria	Ammonifying bacteria
53	In soil, which one of the following bacterial genera is responsible for degradation of cellulose	Escherichia	Pseudomonas	Salmonella	Staphylococcus	Pseudomonas
54	Which one of the following compound is known as the most resisant to microbial degradation during organic matter decomposition	cellulose	chitin	hemicellulose	lignin	lignin
55	Soil microorganisms influence above ground ecosystems by contributing to except which one of the following	plant nutrition and health	soil fertility	soil structure	soil texture	soil texture
56	Mycorrizha is a symbiotic	Crick	Fisher	Frank	Funk	Frank

	association between a fungus and the roots of a vascular plant, was first observed by which one of the following scientist					
57	Denitrification is done only by microorganisms, usually by which one of the following	Facultative anaerobes	obligate aerobe	phototrophic aerobe	Microaerophilic	Facultative anaerobes
58	The plant disease control agents inclde to which one of the following microorganism, except?	Ampelomyces quisqualis	Bacillus subtilis	Trichoderme sp.	Bacillus anthrax	Trichoderma sp.
59	In plants, the strains of which one of the following bacterium initiates to the formation of galls?	Agrobacterium	Rhizobium	Pseudomonas	Ralstonia	Agrobacterium
60	In 1888, a dutch microbiologist Beijerinck succeeded in isolating which one of the following bacterial strain from root nodules	Bradyrhizobium japonicum	Rhizobium leguminosarum	Sinorhizobium meliloti	Both a and b	Rhizobium leguminosarum
61	Ammonia produced in the bacteriod needs to be transported to the plants through which one of the following membrane	lipid membrane	periplasmic membrane	symbiosome membrane	plasma membrane	symbiosome membrane
62	Pyrethrin is got from	Azardiachta indica	Urtica dioca	Tagetus erecta	Chrsanthemum cinerarifolium	Chrsanthemum cinerarifolium
63	Which one is green manure/biofertilizer	Sesbania	Rice	oat	Maize	Sesbania
64	Azolla is used as biofertilizer as it has _____	Rhizobium	Cyanobacteria	Mycorrhiza	Large quantity of humus	Cyanobacteria
65	Green manuring increases the crop yield by	5-10%	15-25%	30-50%	80-90%	30-50%