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**Id 16150**

**Assignment" Fundamental  
Microbiology-II "**

Note:

Attempt all questions from this section, all questions carry equal marks.

Answer Briefly and to the point, don't cut past avoid un-necessary details

**"Enumeration"**

- Enumeration in microbiology is the determination of the number of microbes in a sample
- It is also often essential to determine the number of microorganisms in a given sample.
- For example, the ability to determine the safety of many foods and drugs depends on knowing the levels of microorganisms in those products.

The presence of coliform bacteria, specifically E. coli (a type of coliform bacteria), in drinking water suggests the water may contain pathogens that can cause diarrhea, vomiting, cramps, nausea, headaches, fever, fatigue, and even death sometimes.

### **Microorganisms found in soil are:**

Microorganisms which live in soil are algae, bacteria, actinomycetes, bacteriophages, protozoa, nematodes and fungi

#### **"Algae"**

They play a variety of roles in soil. One of the important role of blue-green algae is that it has revolutionised the field of agriculture microbiology due to use of cyanobacterial biofertilizer.

#### **"Bacteria"**

The number and types of bacteria are influenced by soil types and their microenvironment, organic matter, cultivation practices, etc. They are found in high number in cultivated than virgin land, maximum in rhizosphere and less in non-rhizosphere soil possibly due to aeration and nutrient availability.

**Based on regular presence, bacteria are divided into two groups:**

(a) Soil indigenous (i.e. true resident) or autochthonous, and

(b) Soil invader or allochthonous.

### **"Actinomycetes"**

Actinomycetes share the characters of both bacteria and fungi, and they are commonly known as "ray-fungi" because of their close affinity with fungi. They are Gram-positive and release antibiotic substances.

### **"Bacteriophage"**

Bacteriophages as well as plant and animal viruses have been observed in the soil. However, their role has not been clearly understood.

### **"Protozoa"**

In moist soil most of the members of microfauna remain in encysted form. The population of each group is  $10^3$  per gram wet soil. The role of soil protozoa is predatory, as they eat upon bacteria and thereby regulate their population.

### **"Nematodes"**

Until the role of nematodes in soil was understood nematology was in its infancy stage. In recent years the ecology of nematodes has been greatly advanced. Nematodes derive nutrients for their growth and reproduction from the cell contents and cytoplasm of protozoa, bacteria, fungi, etc. Some common examples of protozoa are Colpoda, Pleurotricha, Heteromita, Cercomonas, Oikomonas, Phalansterium, etc.

## "Fungi"

In most of aerated or cultivated soils fungi share a major part of the total microbial biomass because of their large diameter and extensive net work of mycelium. However, population of soil fungi ranges from  $2 \times 10^4$  to  $1 \times 10^6$  propagules per gram dry soil and its number differs according to isolation procedure and composition of media.

## Microorganisms found in milk are:

Common psychrotrophic bacteria in milk are species of Micrococci, Bacilli, Staphylococci, Lactobacilli, Pseudomonas, and coliforms. Pseudomonas species are the most common and typically have the most impact on quality.

Differentiate between

- **Nitrogen and phosphorus cycle**

1. Nitrogen is recycled where as phosphorus is not
2. Animal get most of their Nitrogen for the water they drink whereas they get their phosphorus from the food they eat.
3. Nitrogen occurs primarily in deep sediments whereas phosphorus occurs primarily in the atmosphere.
4. Nitrogen is lost to the oceans whereas phosphorus is not.
5. Nitrogen has a gaseous phase whereas phosphorus is not.
6. Both phosphorous and nitrogen are essential for plants and animals. These form their respective biogeochemical cycles that show the movement of nitrogen and phosphorus through the lithosphere, hydrosphere an biosphere. However, the atmosphere does not play a major role in the movement of phosphorous.

## "Foodborne infection "

1. Foodborne infection is caused by the ingestion of food containing live bacteria which grow and establish themselves in the human intestinal tract.
2. For a foodborne illness (poisoning) to occur, the following conditions must be present:
3. The microorganism or its toxin must be present in food.
4. The food must be suitable for the microorganism
5. to grow.
6. The temperature must be suitable for the microorganism
7. to grow.
8. Enough time must be given for the microorganism
9. to grow (and to produce a toxin).
10. The food must be eaten.

## "Foodborne intoxication"

1. Foodborne intoxication is caused by ingesting food containing toxins formed by bacteria which resulted from the bacterial growth in the food item.
2. The live microorganism does not have to be consumed.
3. Intoxication: occurs from eating a food that contains a toxin produced by bacteria
4. . An example of food intoxication is Clostridium botulinum poisoning.
5. Intoxification: occurs when live bacterial cells are ingested which then produce toxins in the body. An example of food intoxicification is Clostridium perfringens.
6. Types of intoxications:
  - Bacterial intoxication
  - Fungal intoxications
  - Chemical intoxications
  - Plant toxicants
  - Poisonous animals

"Differentiate between Fermentation and Pasteurization"

- **"Fermentation"**

1. Fermentation for food (again for milk, but there are other kinds of fermentation) is a method for allowing certain "good" bacteria to grow so that they can begin eating the natural milk sugar (lactose) in milk and turn it into lactic acid (to make yogurt, etc).
2. Fermentation is the chemical breakdown of a substance by bacteria, yeasts, or other microorganisms, typically involving effervescence and the giving off of heat.
3. Fermentation for food is a method for allowing certain good bacteria to grow so that they can begin eating the natural milk sugar in milk and turn it into lactic acid.





## "Pasteurization"

1. Pasteurization is the process of heating the object whatever it is for example milk it is heated then the harmful things are killed and made safe..
2. Pasteurization is method of heating of milk at high temperature and then quickly cooling it..
3. Pasteurization or pasteurisation is a process in which water and certain packaged and non-packaged foods (such as milk and fruit juice) are treated with mild heat, usually to less than 100 °C (212 °F), to eliminate pathogens and extend shelf life.

### ● Settle plate :

- Are used to force air into, or onto its collection medium (e.g., Petri Dish with nutrient agar based test media) over a specified period of time.
- Settle plates (Petri dishes) are opened and exposed to the air for specified periods of time to determine what microbiological particles may be present in the environment, as they may settle out of the ambient air, and onto the media surface of the Petri Dish.. These plates are then incubated and analyzed

## **Slit Sampler:**

Comparing settle plates with the Active Casella Slit Sampler, while Sayer et al. did not find this correlation using the Andersen Active Sampler, and Petti et al. demonstrated that, at low air contamination levels, results provided by active Surface Air System sampler (SAS) and settle plates were not correlated. Additionally, it could be interesting to also study the bio-contamination before the start of the operation (at rest) when the room is empty, as the ISO norm suggests, in this way checking the performance capabilities of the theatre, especially its air systems.

- **"Ultrafiltration and Nano filtration"**

## **"Ultrafiltration"**

1. Ultrafiltration has a pore size of approximately 0.002 to 0.1 microns,
2. UF will remove all microbiological species removed by MF (partial removal of bacteria), as well as some viruses (but not an absolute barrier to viruses) and humic materials.
3. Disinfection can provide a second barrier to contamination and is therefore recommended.

## "Nano filtration"

### 3) NANOFILTRATION

1. Nanofiltration membranes have a nominal pore size of approximately 0.001 microns
2. Pushing water through these smaller membrane pores requires a higher operation pressure than either MF or UF.
3. These systems can remove virtually all cysts, bacteria, viruses, and humic materials.
4. NF membranes also remove alkalinity, also removes hardness from water, which accounts for NF membranes sometimes being called "softening membranes."
5. Hard water treated by NF will need pretreatment to avoid precipitation of hardness ions on the membrane. However, more energy is required for NF than MF or UF.

Q3: (10 Marks)

- a) What do you know about Giardiasis?  
What are the difference between  
Cyst and trophozoite form of Giardia

Ans: **Giardiasis**

- Giardiasis is a diarrheal disease caused by the microscopic parasite Giardia.

#### **Organism and Disease Associations**

- Giardia lamblia, Giardia duodenalis, Giardia intestinalis
- Giardiosis (back-packers diarrhea), beaver fever

#### **• Hosts and Host Range**

- Man is the main reservoir
- others, dog, cats, cattle (reservoir),

#### **Geographic Distribution and Importance**

- Cosmopolitan
- Prevalence - 2-5% in industrialised countries
- 20-30% in developing countries
- Most commonly reported human intestinal parasitic infection
- Sporadic individual infections / epidemic form (drinking water).

#### **Difference between Cyst and trophozoite form of Giardia.**

Cysts are smaller than trophozoites, measuring 40-60 mm across. Cysts are round and have a tough, heavy cyst wall made of one or two layers. Usually only the macronucleus and perhaps cilia and contractile vacuoles are visible in the cyst.

## **Trophozoite**

**Size** 50-130 mm long by 20-70 mm wide

Giardia lamblia exists in two forms, an active form called a trophozoite, and an inactive form called a cyst. The active trophozoite attaches to the lining of the small intestine with a "sucker" and is responsible for causing the signs and symptoms of giardiasis.

## **Cyst**

**Size** 40-60 mm across

Giardiasis (gee-ar-die-a-sis with a soft "G") is an infection of the small intestine that is caused by the parasite, Giardia duodenalis, also known as Giardia lamblia and Giardia intestinalis. ... Cysts of Giardia are present in the feces of infected persons.

b) What are the differences between erythrocytic and ex-erythrocytic stages of malaria parasite?

"Erythrocytic "

- Within the red cell the parasite grow in a membrane bound digestive, vacuole, hydrolyzing haemoglobin through secreted enzymes.

A) Continuation of asexual reproduction; Most of the parasites develop into

**Trophozoites Schizont :**

Multiple chromatin in

Schizonts develop into

**Merozoite**

Red cell lysis or merozoites

infect other red cells

B) Production of gametocytes.

Some parasite develop into

sexual forms called

**Gametocytes**

Infect the mosquito.



## Ex erythrocytic stage:

- Parasites inside hepatocytes  
**Shizont**(containing thousands of merozoites) infected hepatocytes release about 30,000 **Merozoite** (asexual, haploid form) quick infect red cells)
- [*P. Vivax and P. ovale form Latin hapnozoides in hepatocytes, which cause relapse of malaria long after initial infection*]

### Q4: (10 Marks)

- a) What are importance of transformation, transduction and conjugation?

## Ans": Importance of transformation "

- In transformation, a bacterium takes in DNA from its environment, often DNA that's been shed by other bacteria.
- If the DNA is in the form of a circular DNA called a plasmid, it can be copied in the receiving cell and passed on to its descendants.
- Transformation of cells is a widely used and versatile tool in genetic engineering and is of critical importance in the development of molecular biology.
- The purpose of this technique is to introduce a foreign plasmid into bacteria, the bacteria then amplifies the plasmid, making large quantities of it.



## "Importance of transduction"

- Transduction is the process by which DNA is transferred from one bacterium to another by a virus.
- In transduction, viruses that infect bacteria move short pieces of chromosomal DNA from one bacterium to another "by accident."
- Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA
- Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome
- The viruses that infect bacteria are called bacteriophages.
- When one of these "defective" bacteriophages infects a cell, it transfers the DNA.

## "Importance of Conjugation"

- Conjugation is the process by which one bacterium transfers genetic material to another through direct contact.
- During conjugation, one bacterium serves as the donor of the genetic material, and the other serves as the recipient
- The donor cell close itself to the recipient using a structure called a pilus, DNA is transferred between cells.
- In most cases, this DNA is in the form of a plasmid.

b) Compare and contrast between lytic and lysogenic cycle?

Ans: "Lytic cycle"

- Viral DNA distroys cell DNA takes over cell function and distroy cell
- The virus replicates and reproduce
- There are symptoms of viral infection

## "Lysogenic cycle"

- Viral DNA merges with the cell DNA and does not distroy the cell.
- The virus does not reproduce
- There are no symptoms of viral infection

### Q5: (10 Marks)

**How many type of Blood smear we formed?  
Write down procedure of Leishman staining?**

**Ans:** A blood smear examination: Compares the WBCs' size, shape, and general appearance to the established appearance of "normal" cells. It also determines the five different types of WBCs and their relative percentages (manual WBC differential).

#### **Ans procedure**

1. Pour Leishman stain dropwise(containing the drops on the slide and wait for 2 minutes. This allows fixation of the PBF in methyl alcohol
2. Add double the quantity of buffered water drop wise over the slide.
3. Mix by rocking for 8 minutes. Wash on water for 1 to 2 minutes.
4. Dry in air and examine under oil immerdion lens of thr microscope

