COURSE TITLE: GENERAL MICROBIOLOGY II

COURSE CODE: MCB 202 (3 UNITS)

TOPIC: **STUDY OF ECOLOGICAL DISTRIBUTION OF MICROORGANISMS IN DIFFERENT ENVIRONMENTS AND THE SPECIFIC PROPERTIES THAT ADAPT THEM TO THEIR PARTICULAR ECOLOGICAL NICHES**

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MICROBIAL ECOLOGY

General Introduction

In this course, we shall consider the existence of microorganisms as exist in communities. Recent estimates suggest that most microbial communities have between 10^{10} to 10^{17} individuals representing at least 10^7 different taxa. How can such huge populations exist and moreover, survive together in a productive fashion? To answer this question, we must know which microbes are present and how they interact. That is, we need to study microbial ecology, a multidisciplinary field which discusses some aspects of environmental microbiology.

Microbes deal with small or minute organisms, while ecology is a branch of biology dealing with organisms, their relationship with one another and that of their environment. There are several approaches to the teaching and learning of microbial ecology. One approach is to consider the activities of the microorganism in nutrient cycles and food chains; another approach is to look at it from the factors which govern microorganisms and their environments.

Generally speaking, microbial ecology is seldom primarily concerned with the organism *per se* rather it is concerned with their activities in terms of the resultant chemical and physical changes they make in the environment and those aspects of nutrient cycles, polluted water sources, diseased patients, biochemical capacities of causative organisms to induce infection and so on are all considered as aspects of microbial ecology.

General Objectives

The objective of this course is to study microbial community dynamics and the environments in which they live.

For the purpose of this course the soil, air, water, animal and plant surfaces are considered as specific environments.

UNIT 1. MICROBES AND ECOLOGICAL THEORY

Introduction

In this unit, we shall define some basic terms used in microbial ecology. We shall also consider colonization and succession as natural phenomena in ecosystems. Some specific examples are presented.

Objectives

The objectives here is to equip students with the appropriate use and application of some basic ecological terms; and to enable us understand that colonization and succession are natural events which progress from a freshly exposed surface to a pioneer community and finally terminate in a climax community.

Main Contents Basic Definition in the Study of Microbial Ecology

1. **Ecosystem**: This is a combination of biotic and abiotic components of a specific environment. Normally, such a unit has a collection of organisms and abiotic components which are unique to it and as such, one eco-system is different from the other.

2. **Community** This is a collection of microorganism inhabiting a given site in the ecosystem (that is the ecosystem without the environmental factors or abiotic factors).

3. **Population**: This is the individual microbial species. Unlike the community, it is more homogenous and specific.

4. **Individual**: This refers to the individual organisms in the population. Each ecosystem has, associated with it certain physical, chemical and biological characteristics. These factors govern the composition of the community by dictating which of the individual microorganisms will be successfully established, and out of those established, some will be dominant while others are limited and other groups are totally eliminated. Therefore, selection by environmental factors is important. Those organisms that are established are those that are better adapted to the abiotic conditions (P^H, temperature, moisture, water activity, oxygen concentration etc) in that particular environment.

Organisms that cannot cope with the prevailing conditions are eliminated. The environment builds the community through selection. It is as a result of this relationship with a community that we have certain Species of micro-organism as dominant.

The Habitat: A habitat is an area having a degree of uniformity in terms of the abiotic components. They are therefore also considered to be of ecological significance e.g. surfaces of plants and animals, soil, open sea, blood, air, nasal passages, alimentary cannal and so on. The size of the habitat is not important because it varies considerably. The important factor is that certain sets of conditions are uniform to that area.

Niche. The ability of microorganisms to make use of resources available in their habitat is varied and this has lead to the idea of niche. The role of a particular organisms in a particular place is the niche.

Microorganism could be divided into types depending on their functions in the different habitats, those with narrow and those with broad range of tolerance. Those with narrow niche are highly specialized and perform single function or role e.g. obligate parasites, autotrophic organisms, while those that carry out a range of functions are said to have a broad niche e.g. heterotrophic organism. Those that occupy a narrow niche are easily eliminated if there is a change in environmental conditions especially as it affects their survival, but they however, flourish luxuriantly when their conditional requirements are met. Examples are photosynthetic micro-organisms which are adversely affected when the source of light is blocked but they grow rapidly when there is light. On the other hand, those that have a broad niche are not severely affected by changes in the environmental conditions.

1.2 COLONIZATION AND SUCCESSION.

When an area is denuded or freshly exposed e.g. when a tissue is freshly exposed or wounded or when we have an earth quake which exposes the earth's surface, a number of microorganism will be deposited on such surfaces. The first microorganisms to arrive on such surfaces are called **pioneers**. These organisms grow and multiply to form the pioneer community and from the time the exposed areas are occupied by microorganisms, it is said to be **colonized.**

After the establishment of the pioneer community, they feed on the substrates, produced byproducts and other waste materials or metabolites and so the environment becomes modified. The modified environment paves way for colonization by other organisms, while the pioneer communities are gradually eliminated. Thus we have a situation whereby one community will out-grow and replace another. This phenomenon is referred to as **succession.**

1.3.0. SUCCESSION IN NATURAL HABITATS

1.3.1 SUCCESSION OF MICRO-ORGANISMS ON DUNG.

If a fresh dung is placed under a bell jar and a fairly suitable humidity is maintained, a succession of fungal fruitifications can be observed. The first to appear are Zygomycetes followed by Ascomycetes and then Basidiomycetes. Each group has been considered to represent sugar, cellulose and lignin utilizers respectively.

Harper and Webster (1964) while confirming the sequence have shown that it is not a succession based on nutritional factors. They grew a number of fungi involved under a variety of conditions and showed that each group had a characteristic minimum time before commencement of growth and appearance of fruiting bodies. If the fungi are listed in the order based on the minimum time, the sequences is also found to be the same as on dung.

According to these authors, the succession of fruiting bodies on dung is connected with the duration of necessary developmental periods rather than different assimilatory abilities.

1.3.2 COLONIZATION OF STERILE HUMAN HAIR.

Griffin (1960) made a sequential study of sterile human hair placed on the surface of various soils. In general, his observations showed that the first colonizers where *Fusarium* species, *Penicillium* species and some *Mucorales*. These species rely on simple sugars. The second group of colonizers includes *Chaetomium cochloides, Humicola* species, *Gliocladium roseum* and *Penicillium* species. The first two are cellulolytic while the last two are polysaccharide users.

The third group are keratinolytic members of Gymnoascaccae which are mainly Ascomycetes. In this case, succession is based on nutrition. This is more clearly shown in the late predominance of species utilizing the most resistant component of the substrate (Keratin).

UNIT 2: MICROORGANISMS IN ECOSYSTEMS

Introduction

In this unit, we shall consider the various environments where microbes can exist. The microbial environment is complex and constantly changing. It often contains low nutrient concentrations (oligotrophic) and exposes microbes to many overlapping gradients of nutrients and other environmental factors. The physicochemical factors that govern the growth and survival of microbes in these environments.

Objectives

The objectives of this unit are to consider the various microbial environments, survey the effects on microbial growth of individual environmental factors such as water availability, p^H, temperature, oxygen concentration etc.

Main Contents 2.1.0 SOIL MICROBIOLOGY

The soil (as an ecosystem) is the natural medium for terrestrial plant growth. It is composed of varying proportions of organic and inorganic components which arise as a result of interactions between many complex processes such as weathering of rocks, decomposition of plants and animals materials, and redistribution of materials by water movement and human activities. Some of the particles forming soil come together to form aggregates or clumps.

The way in which they are arranged spatially gives the soil a structure.

Like many other types of environments, microorganisms (bacteria, fungi, algae, nematodes, actinomycetes, and protozoa) are also present in the soil and they are known to perform various functions and even contribute to the structure of the soil by producing gums and cement which apart from holding the soil together can also damage the structures by blocking the pores.

Functions of soil Fungi: Fungi are abundant in the soil, however not as bacteria. Different soils have different types of fungal species associated with it depending on the prevailing conditions. Fungi are active in the soil as mycelia and are usually dormant as spores.

1. One of the most important functions of soil fungi is the degradation of complex plant structures like hemicelluloses, pectin, lignin, cellulose etc into simple molecules which are made available to plants as nutrients.
2. They help in the formation of stable soil by binding the soil through hyphal penetration.
3. Fungi are able to breakdown complex proteinous materials, producing ammonia and sulphur compounds which could be used by higher plants.
4. Fungal degradation activities improves soil texture and organic composition
5. Soil fungi may function as parasites in the soil under certain conditions to the disadvantage of higher plants
6. Often, fungi compete with higher plants for nutrients like nitrates and ammonia.
7. Under certain conditions, they are able to trap nematodes and protozoa in the soil (biological control).

Soil Bacteriology: Bacteria are known to be the most abundant type of soil micro-organisms. Hundreds of species of bacteria are present in 1gram of soil and they vary in different soils found in various parts of the world. The nature and extent of soil bacterial population however depends on prevailing environmental conditions, such as moisture, p^H, temperature, aeration and nutrient availability. Soil fertility is largely dependent on bacterial activity, and soil bacteria are essential to all life processes because without putrification and decay, there will be no decomposition of dead plants and animal matter.

Soil bacteria by their activities also make simple chemical substances such as Nacl, $Capo_4$, $NaNo_2$ etc available to plants.

2.1.1 FACTORS AFFECTING MICROBIAL GROWTH IN THE SOIL (Soil as an environment for microorganisms).

A combination of a number of factors physical, chemical and biological make it possible for microorganisms to survive in the soil.
Among these factors are:

a. Soil components (soil type and structure)

b. Soil water/moisture content

c. Aeration

d. Soil temperature

e. Soil p^H

f. Soil profile

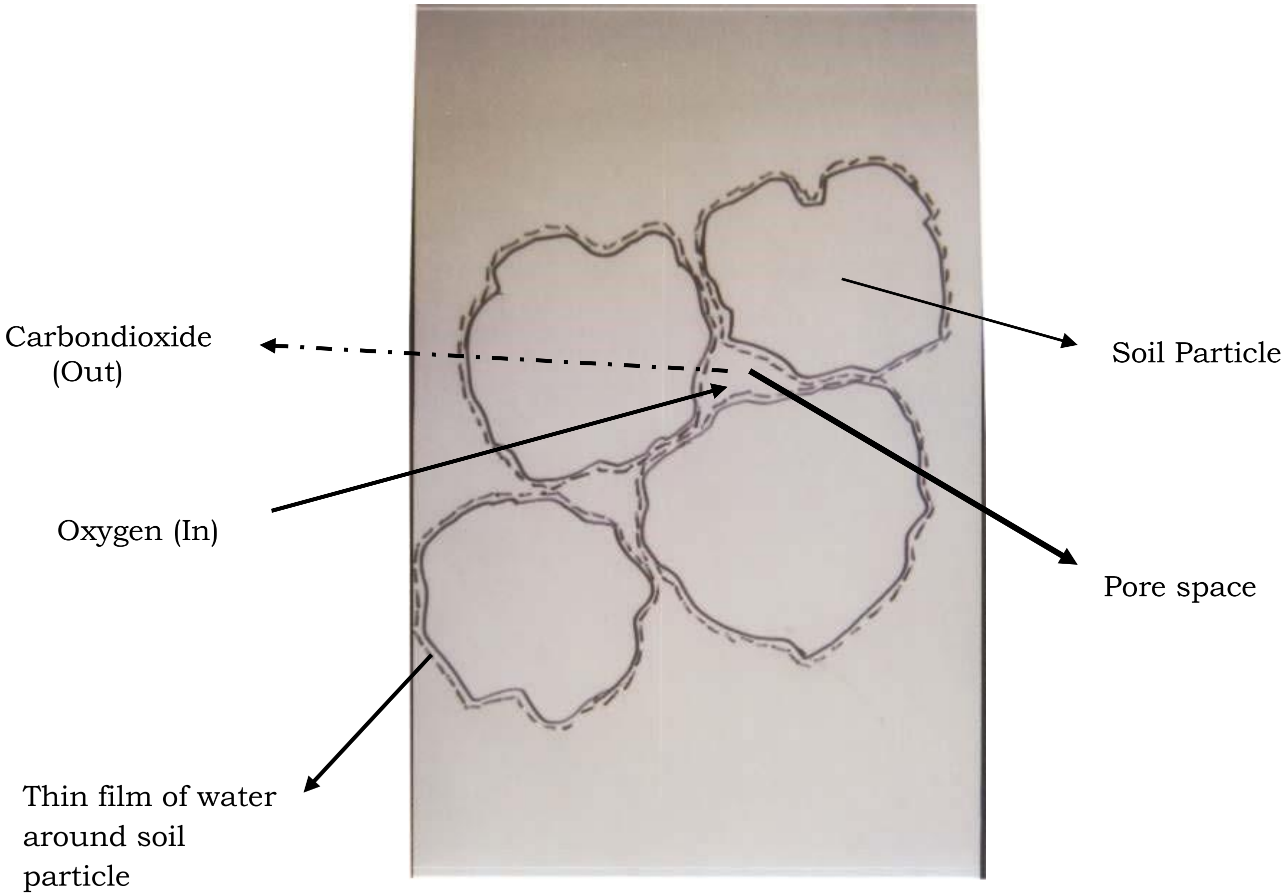
Most of these components are constantly changing, and fluctuations in one component may result in fluctuations in the other factors. Thus, an alteration in moisture content influences aeration, temperature, and chemical reactions in the soil.

Soil Components: The soil consist of a mixture of weathered rock of various sizes. The proportion of the size determines the soil texture. Clay particles are important components of soil environment; and they greatly influence other physical and chemical properties of the soil. Montmorillonite (a clay mineral) when present in the soil in low concentrations reduces the rate of the fungal respiration while stimulating bacteria either at low or high concentrations. This type of mineral preserves a p^H condusive to bacterial growth. Organic matter and humus are formed from remains of plants and animals remains and are constantly supplied to the soil. Organic matter which is not completely decomposed contributes to the formation of amorphous humic materials which is usually important in soil studies. Humus is not a chemically defined entity. It is a complex mixture of many substances the bulk of which is insoluble dark colored materials. Generally speaking, organic materials in the soil decrease with soil dept.

Soil water/moisture content: Dissolve a known weight of soil in a known volume of water (usually, soil: water in ration 1:2); dry to constant weight and then:

$$\text{Moisture Content} = \frac{\text{less in weight on drying}}{\text{initial weight}} \times 100\%$$

Soil water is usually present as a thin film around the soil particles.



Aggregates of Soil Particles

Generally, soil water is subject to extreme fluctuations and this has some important effect on soil microbial populations. Many microbial cells are killed by desiccation and only those ones with resistant structure can survive drought. Warcup (1957) found out that fungal species survive in Australian soils as spores, rhizomorphs, sclerotia and resting hyphae. Based on soil water, microbial populations are known to fluctuate with the seasons of the year. Studies have also shown that remoistening dry soil results in the restoration of microbial activities. This is due to the fact that when water is available we have more soluble nutrients becoming available to microorganisms. On the other hand, drying of soil is known to kill some microorganisms which then produce a source of nutrients for the survivors.

Drying could also result in the evolution of certain types of microorganisms which can adapt themselves to low water requirements.

ROLES OF SOIL WATER

- Water is useful in keeping microbial cells turgid.

- Water plays some roles in dispersal of spores.
- Water provides a medium for movement of reproductive structures in some species.
- Water is useful in the diffusion of toxic metabolic wastes away from the cells.
- Soil water is also useful in the distribution of extracellular enzymes in the soil.
- Water is a universal solvent and it is the site and medium for organic reactions.
- Soil microorganisms live in the thin film of water around soil particles.

Soil Aeration

The amount of air present in the soil varies, and this often has some relationship with soil water content. There is also an exchange of gases between the soil and the atmosphere through the air spaces in the soil. The amount of air present the soil varies with the soil type and the different points in the soil e.g in regions of intense microbial activity, especially near plant roots (rhizosphere) the concentration of the air will be drastically altered as a result of metabolism by rhizosphere organisms.

Microbes very considerably in their responses to changes in the concentration of air in the soil and atmosphere. Some bacterial species are strict anaerobes (e.g. *Clostridium botulinum* (food poisioning) while some are aerobic (e.g. *Pseudomonas fluorescence*). Most fungal species are aerobic.

Decomposition of substrates under anaerobic condition by microbes may result in the formation of incompletely oxidized end product e.g. when cellulose is decomposed by bacteria in aerobic condition the end products are $C0_2$ and H_20; but under anaerobic condition oxidation is incomplete and organic acids such as acetic acid accumulates and this in turn affects soil p^H. The concentration of oxygen in the soil also affects the oxidation reduction in the soil while concentration of $C0_2$ affects soil p^H,

- provides a source or environment for autotrophic microbes, and
- have some effect on heterotrophic micro-flora. The deeper you go into the soil there is a decrease in soil aeration.

Soil hydrogen ion (H^+) concentration

Suspend 5 grams of soil in 10 mls of water and mix thoroughly. Take the p^H using a p^H meter or litmus paper. Based on the p^H tolerance of organisms, soil microorganisms have been classified into:

Acidophiles – low p^H lovers

Alkalinophiles – high p^H lovers

Halophiles - grow in high salt concentrations.

The soil p^H has remarkable influence on soil microorganisms. Soil p^H can be affected by a number of factors such as the salt concentration and concentration of $C0_2$ in the soil. Thus as these factors fluctuate soil p^H also changes.

Leaching also affects the soil p^H. As water moves through the soil, bases are leached out and replaced by H^+, therefore constant soil leaching leads to the formation of acid soil. Such changes can also affect other physical properties of the soil.

Majority of soil microorganisms grow well at p^H of 7. However, there are a number of exceptions especially *Thiobacillus* species. This organism is acidophilic and can grow at a p^H of 0.6. Generally, soil bacteria and actinomycetes are less tolerant to acid conditions than fungi. Many species of fungi grow in acid conditions and acid soils such as Pod-sols where the p^H may be as low as 3.0. However, p^H preferences are varied among genus and even species. It should however be noted that the occurrence of a microbe in soil with certain p^H does not mean the particular microorganism is carrying out all its activities at that p^H.

Decomposition and other microbial activities taking place on the soil surface result in the release of acids and ammonia and these in turn results in an increase in the p^H of the soil surface

Soil temperature: Use a good thermometer (Thermocouple) to record temperature. Based on temperature requirements, soil microbes have been classified into

Psychrophiles – cold lovers (0-20^{0}c)

Mesophiles – moderate temperature lovers (20 – 40^{0}C)

- Optimum at 25^{0}c – 37^{0}c

Themophiles = High temperature lovers.

- 20 – 50^{0}c
- Optimum around 40^{0}c

Thermotolerants are mesophilic but capable of tolerating high temperature conditions.

The sun is the major source of soil temperature and several factors are responsible or determine the solar energy absorbed by the soil. Among there factors are:

- The angle at which the sun hits the land.
- The color of the soil; (light colored soils absorb heat less than dark colored soils);
- Vegetation cover; and
- Amount of soil litter

The average monthly temperature on the surface of forest soils varies from 2-19^{o}c, and at 7.5cm depth, it is reduced to 4-14^{o}c. There is a close association existing between the soil moisture content and the heat absorbing capacity. About one calorie of heat is required to raise one gram of water to 10^{o}c while 0.2 calorie is required to raise 1gram of dry soil to 10^{0}c. This implies that the thermal conductivity of wet soils is higher. As soil temperature rises so also the diffusion rate of gases rises. Therefore this results in a complex interaction between moisture, temperature and aeration. The higher its temperature, the more the microbial activities and the more the microbial population. Temperature requirements however varies among the different groups of microbes.

Soil profile/depth. This denotes changes as you go into the soil. Microbial populations in the soil decreases with increase in soil depth. That is, on the soil surface, a lot of microbial activities are taking place. Therefore the population of microorganisms in this zone is usually higher. Other reasons to explain these trends include:

- Presence of substrates which act as food for the microorganisms on the surface layer.
- The effect of sun rays on surface soil temperature has some relationship with enzymatic activities.
- Because of agricultural activities and microbial interactions, soil particles on the surface are not too closely held together and this gives room for aeration

- The water content in soil surface contributes to microbial interactions

2.2.0 AEROMICROBIOLOGY.

This is the study of micro-organisms in the air. Like the soil and other environments, micro-organisms are represent in the air and perhaps because of their role in the transmission of some air-borne infectious diseases and the dispersal of microorganismKs, the study of aeromicroorganisms has been receiving attention from microbiologists and scientists generally. The components of the aerial environment are varied and mostly include pollen grains, dust particles, fungal spores, bacterial spores, fungal hyphal fraqments, actinomycetes, spores of bryophytes and pteridophytes e.t.c

Usually, micro-organisms in the air are in a state of suspended animation. Many of them are easy killed by desiccation, ultraviolent rays and other unfavorable conditions. Therefore the living micro-organisms are either usually resistant or have been in the air for some time. Resistant spores are capable of producing pigments and other means of adaptations.

The dispersal of air – borne particles involves 3 stages

- Liberation and take off into the air
- Dispersion in air current
- Deposition on surfaces at the end of the journey before germination and growth.

LIBERATION:- Before a particle becomes airborne, a number of problems have to be overcome. Example, energy is to required to overcome the adhesive forces attaching the particles to the surface of the substrates; also the particle has to be of a size that will be airborne. The degree of adaptation to air borne dispersal varies greatly between different groups of micro-organisms and this is reflected in their relative abundance in the air.
Viruses and bacteria are poorly adapted while fungi have developed many adaptations mechanizing to enable their spores become readily air-borne. While some have long sporophores (stalk) which lift their spores well into the air, some others have ways of forcefully ejecting their spores into the air. Some others rely on passive means. Rain splash, mechanical disturbance and other physical adaptations help a lot in spore liberation into the air.

Dispersion:- The dispersion of air-borne micro-organisms can be considered at two levels.

Fate of individual spores; and the behavior of groups or cloud of spores.

These aspects are related and depend on the physical characteristics of the spores and that of the atmosphere. The important characteristics of spores in this respect are size, shape, degree of surface roughness, density and electrostatic charges; while those of the environment include wind movements, turbulences, layering convention, wind gradient near the ground and the pattern of atmospheric circulation. Because they are heavier than air, spores tend to settle under gravity but because they are also blown by air, they are affected by electrostatic charges. The distance traveled by a spore depends on the interrelationship of the various factors mentioned.

Deposition: - The final stage of airborne dispersal of microorganisms is deposition. The microbes are returned to the surface layer of plants, animals or soil so that they can no longer be blown by normal wind, though they may still be washed off. Deposition may occur in precipitation or from dry air by several different methods such as sedimentation, impaction, rain washing and so on.

2.2.1 FACTORS AFFECTING CONCENTRATION OF MICROORGANISMS IN THE ATMOSPHERE.

A number of factors can directly or indirectly affect the amount of micro-organisms and their survival structures in air. Such factors may include wind speed, rain, temperature, pigmentation, humidity, vegetation and human activities.

Wind Speed. Fast moving air blows force with it and so readily provides energy for detachment of spores from their vegetative structure and other surfaces. Usually, it is difficult for spores to be deposited at a spot except where there is an obstacle or a wind breaker along the wind course.

Rain Splashes. Rain washes air-borne spores to the soils and as such the amount of rain received by an area may have some influence on the number of air-borne spores and microorganisms. Where an area has a clearly defined raining and dry seasons, the amount of dust particles and spores in the air may vary with the seasons of the year.

Temperature. During the dry season, those air-borne spores that are thin walled are easily dehydrated to the advantage of those with thick warty walls which are more adapted to survive high temperatures. There is also a relationship between temperature and humidity, then temperature and wind movement. Freezing temperature is likely to be encountered by air-borne microbes above 3-5 km in the air (the higher you go the cooler). Depending on how well adapted the structures of the microbes are, the very low temperatures may or may not have a damaging effect on the survival structures of micro-organisms.

Humidity. The water amount in the air is usually referred to in terms of relative humidity that is the ratio of the actual vapour pressure when the air is saturated with water at the same temperature. Vapour pressure varies greatly with temperature and thus relative humidity is low when temperature is high and vice- visa. Relative humidity is a good indicator of the drying effect air on micro-organisms. A wide range of relative humidity may be founds in the atmosphere from 10- 20% in desert regions to saturation or super saturation where cloud formation occurs at ground level resulting in dews or frost.

Different micro-organisms have different relative humidity requirements for their survival in the air. The lower limit of relative humidity for fungal growth is about 65%, while bacterial species require more.

Pigmentation and ultra-violent rays. Ultraviolent radiation in the air originate from the sun much of which is reflected or absorbed before reaching the earth's surface. Micro-organisms carried into the higher regions of the atmosphere may be exposed to the damaging doses of U.V radiation. Though ultra-violent radiation of about 265mm is most lethal, other short waves radiation even within the visible region can also be damaging. Pigmented spores especially those of dermatophytes can resist strong ultra-violent rays from the sun. Generally speaking, radiation, desiccation and temperature all interact to affect the survival of micro-organisms in the air. The manner of interaction is not well understood but desiccation and freezing may protect micro-organisms against radiation damage.

Vegetation: This can affect spores in air in two different ways. Leaves can trap spores when washed down by the rain. Some may germinate when conditions are suitable. On the other hand, when the leaves dry off the spores can be blown back into the atmosphere and thus increasing the number of air-borne spores.

Man's Activities: As a result of poor drainage systems, of rubbish dumps and so on, micro-organisms in rubbish dumps and dry feacal materials are blown up into air. Also during activation and soil digging, soil-borne spores are released into the atmosphere. This may be used to explain why some farmers in some parts of the world suffer from an infection of the lungs and pulmonary tracts caused by *Aspergillus* species, and the disease is called aspergillosis.

PRACTICAL APPLICATION AND IMPORTANCE OF AEROMICROBIOLOGICAL STUDIES.

The application of aero microbiology is of great importance in disease epidemiology, forecasting and modeling. Epidemiology is the study of incidence, distribution and control of diseases within populations or the sum of factors controlling the presence or absence of a disease or pathogen. Epidemiology therefore concerns the interaction between the pathogen, its host and the environment. Aerobiology can make valuable contributions to the epidemiology of animal as well as plant diseases. Sinnecker (1976) recognized six types of diseases with aerogenic transmission depending on:

- the resistance of infective agents in droplets;
- the drop's nuclei or dust;
- the ability to infect from secondary aerosols formed when dust is re-suspended;
- the host range;
- whether there are alternative means of transmission;
- and whether the infective agent can survival for long in droplet form. Even non-infectious diseases can occur epidemically as a result of air-borne infection e.g. coccidiomycosis and histoplamosis, when a population is exposed to large number of causal fungal spores. Aerobiological methods can be used to detect the presence and quantity of infective agents in primary and secondary aerosols. Their persistence in air and relationship with infection can also be known.

In plant pathology, aero microbiological methods have been widely used to determine the number of spores of a plant pathogens in the air. Aero microbiological studies can also be used in forensic and theft detection by plating out pieces of cloths from suspects and matching the microbial isolates with those of the aerial environment where the theft was carried out.

2.3.0. AQUATIC MICROBIOLOGY (Micro-Organism in Natural Waters)

Most microbial environments are aquatic, in that sometime, the vegetative organism live in aqueous media such as animal and plant fluids, soil, water and many other related habitats. Water is also usually required for the movement of various reproductive structures. Aquatic microbiology however refers to that microorganism which live in the earth's natural waters ranging from the small ponds to great oceans. Most of the earth's water is contained in the oceans (97.1-97.6%), while smaller quantities are maintained in polar regions as ice and glaciers (2.1%) and ground water (0.3-0.8%) and in land waters

Lakes	–	0.009% and
Rivers	-	0.00009%

Despite the wide range of conditions encountered in the various aquatic habitats, micro-organisms are found in all types of aquatic habitats.

TYPES OF AQUATIC ENVIRONMENTS

Aquatic environment are often divided into oceans and inland waters. Inland waters can be classified into ground and surface waters. Ground water is that water contained in permeable rocks below the water table. It accumulates as water in the soil perculates through it. Many of the nutrients are therefore filtered out. Because of this, ground water is only able to support a limited population of micro-organisms.

Surface water comprises of lotic or running waters consisting of springs, streams and rivers. The second group are lentic or standing waters comprising of lakes, ponds, swamps and bogs. Springs can occur where ground water breaks the surface and thus like ground water, they are nutrient poor near the origin. Specialized springs occur in certain regions such as hot springs in regions of volcanic activities. We can also have mineral springs and this depends on the surrounding geological conditions. They may occur when water accumulates dissolved mineral as it passes through geological formations. Example hard water and springs found in many lime stone areas. Springs eventually become rivers as their flow is supplemented by tributaries, land run offs and precipitation. A number of factors however determine the rate of flow. Such factor include gradients, transverse conditions of the river valleys etc. Lentic waters differ in size ranging from the smallest ponds to the deepest known lake. Often, distinction between lakes and ponds are not clear but generally, the bottom of ponds are colonized by submerged plants whereas lakes are too deep to be colonized by plants.

The oceans cover approximately 70% of the earth's surface and the depth is variable ranging from 200m to 10000m or more. Because of this great depth much of the sea is in near total darkness. Of great importance to abiotic and biotic components of oceans is the salt content which varies 32% to 38% with an average of 35%. Because of the additional dissolved salts in the sea, the specific heat capacity is high and the freezing point temperature is affected. Salinity is the major phenomenon which is often used to distinguish between water bodies. On this basis, Moore (1961) classified water bodies based on the salt content as follows:

0.5% = fresh water

0.51% = 29.9% = brackish water

30.0% and above = marine (sea and ocean) water

SPECIAL HABITATS IN AQUATIC ENVIRONMENTS

Just like any other habitat, important special habitats can be found in aquatic environments and the micro-organism inhabiting such habitats have special features of survival in such areas. Such feature may be structural or physiological. One of such habitats in any water body is found in sediments and particulate matter comprising the bottom. These vary considerably from sand to mud and silt. These can be classified into three major components according to the origin.

1. **Lithogeneous Components:-** those primarily derived from rock, soil and volcanic ash.
2. **Biogenious Components:-** those composed of skeletal remains of macro- ,and micro-organisms e.g. diatoms
3. **Hydrogenous Components:-** those components resulting from inorganic and chemical reactions occurring in the water. Bottom sediments may be a particularly important reservoir of organic and inorganic nutrients and when highly reduced they may provide habitat for anaerobic organisms. Another importance special habitat in aquatic environments is provided by surfaces of plants, animals and non-biological structures such as stones. Enough bodies

of water surface may be heavily colonized by micro-organisms and physical and chemical conditions occurring at the surface may provide micro-environment much like that of the surrounding water.

Physical and Chemical Factors in aquatic environments

Light: is a critical factors in determining the amount of carbon fixed into an inorganic compound through photosynthesis. Much of the sun's radiation does not reaches the earth's surface since it is absorbed and scattered by atmosphere and cloud cover. Some of the radiation which reach the water surface is reflected back to atmosphere and the small amount that penetrates, suffers further the attenuation through scattering and differential absorption. In pure water, approximately 53% the total light is dissipated as heat and quenched at the first meter of water depth.

In most natural waters, light extinction may be considerably affected by the amount of dissolved solids and suspended materials; and in water with appreciable dissolved and particulate materials, the general effect on light penetration is that there is a general decrease is transmittance. The intensity and specific composition of light penetrating water surface and to any depth has a profound effect on all resident microbial activity since it is the available light which largely determine the potential for productivity.

Generally, photosynthesis increase in relation to light intensity until the photosynthetic system becomes light saturated. Turbidity measurement have been used as a measure of light penetrance, and transparency disc is often employed for this purpose.

Temperature: The amount of heat entering a body of water through insulation depends primarily on the latitude and the water conditions; while the distribution of heat within the water body is dependent on it morphological characteristics and mixing by wind turbulence. In shallow waters such as streams and ponds, there may be large temperature changes. In most case, it is the surface water that is temporarily variable, while deeper waters show alternations seasonally. Such differential heating often can lead to thermal stratification e.g. in a deep lakes, two stratified layers can be recognized viz:

The warm (upper) layer called epilimnion

The cool (deeper) layer called hypolimnion.

However, in shallow lakes there is no stratification because there is a steady mixing of the water. The range of temperature in oceanic surface water is from – 1.7^{o}c in polar regions to 25- 30^{o}c in tropical and subtropical waters. Where as in deeper waters, temperature remains fairly constant at a few degrees above freezing point.

In the oceans and inland waters in cold climate areas where predominant water temperatures are less than 5^{o}c, the resident bacterial are mainly psychrophiles and they can grow event at 0^{o}c. In warmer inland waters, mesophilic bacteria and fungi are the predominant species. Usually if temperature is more than that required for the organisms, the cell components are destroyed or denatured and this results in death. Whereas dropping in temperature below the normal range may not necessarily kill the organisms, it slows down metabolic activity. Within a given range, bacterial metabolism increases with increase in temperature and so there is increase growth with increase temperature, but at temperatures higher than the optimum, growth rate is reduced.
Temperature has a profound effect on enzymatic activity.

PRESSURE. Hydrostatic pressure is not an important factor in inland waters but can be of considerable importance in oceans at great depths. It increases at approximately one atmosphere with 10m increase in depth. Increase pressure affects dissociation constants of carbonic acid and therefore results in a decrease in pH. The pressure requirements of organisms differ greatly even among the species. The term barophilic is used to describe bacteria which grow preferably or exclusively at high pressures; While those which survive at high pressures without injury but are normally active at atmospheric pressure condition are called barotholerant or baroduric.

A number of barophilic bacteria have been isolated from deep seas and from brines in deep oil wells. Most bacteria from surface waters are adversely affected by high hydrostatic pressures but not all bacteria are affected to the same extent. In experiments dealing with bacterial growth rate at high pressures, the relationship between pressure and temperature was established. It was found out that high pressure tend to raise optimum temperature for growth.

DISSOLVED GASES. The two most important dissolved gases in aquatic environments are oxygen and carbon dioxide. Oxygen is impotent because of its importance in aerobic bioprocesses and in relation to oxidation – reduction potentials; while carbondioxide is important for photosynthesis and pH equilibrium. The concentration of oxygen in water is dependent on the water temperature, partial pressure, salinity and biological activities. Oxygen concentration in water is usually expressed in terms of milliliters of oxygen is one liter of water. Carbondioxide it is very important in aquatic environments since it aids the regulation of hydrogen ion concentration. In water, an equilibrium is established between carbondioxide, carbonic acid and bicarbonate as follows:

$$CO_{2+} H_2O = H_2CO_3 \rightleftharpoons HCO_3 + H^+ \rightleftharpoons CO_3^{2-} + 2H^+$$

The solubility of carbondioxide in water is affected by temperature, salinity and other related factors. Apart from these two gases, a number of other dissolved gasses also occur in natural waters e.g. nitrogen which is not utilized by many aquatic micro-organisms but is involved in special microbial processes (nitrogen fixation).
Other gasses produced by microbial activities are methane, H_2, HN_3, H_2S, NH_4 e.t.c.

Hydrogen Ion Concentration (P^H). The optimum P^H for most aquatic bacteria is between p^H6.5-8.5 and this corresponds with the P^H of most large water bodies. The approximate P^H of sea water usually lies below PH 8-8.3 while those of lakes is approximately p^H7; although considerable fluctuations may occur.
There are usually proportionally more fungal species in acid waters compared with neutral and alkaline waters. The effect of p^H is also related to enzymatic activities, and drops in p^H beyond the organism's normal range can affect its physiology, and when extreme, can lead to death. Morphological changes can also occur such as enlargement of cells and irregular swelling and branching.

Photosynthesis is affected by p^H through its influence on carbon dioxide and carbonic acid equilibrium; p^H varies inversely with dissolved carbondioxide concentration and directly with bicarbonate concentration. In aquatic environments, all photosynthetic plants use free carbondioxide with undissociated carbonic acid for photosynthesis. However lack of carbon dioxide at high P^H is not a limiting factor for photosynthesis since plants have enzymes which convert bicarbonates to carbondioxide. Some plants such as *Scenedemus quadricanda* can utilize bicarbonates directly, while *Chlorella pyrenoidosa* cannot.

SALINITY. Though salinity is not itself a physical factor, changes in salinity can have profound osmotic effect which sometimes can be lethal and can also be toxic through denaturation of cellular components. Most marine bacteria are halophytic and some have specific requirements for sodium ions and some will not tolerate too high salt concentrations. Moderate changes in salinity may have morphological and physiological effects e.g. rod-shaped bacteria may form filaments with an increase in salinity and the microbial oxidation of organic acids and sugars are affected

MICROFLORA IN AQUATIC ENVIRONMENT

Micro- organisms present in aquatic environment are mainly phytoplanktons. They include bacteria, algae and fungi; others are protozoa and viruses.

Bacteria: - Usually, the bacterial flora of a river may show a peculiar close relationship with the surrounding terrestrial population because of constant injection of soil, water run offs and organic matters. Some aquatic bacteria are phototrophic while the chemotrophic ones are also widely distributed, but generally the distribution and abundance of the majority of the heterotrophs is largely controlled by the concentration of available organic materials, therefore in a nutrients poor spring, the bacteria present are mainly of Gram negative rods such as *Gallionella, Pseudomonas* etc. Along the cause of the stream, as nutrient content increases, there is a general increase in the number of species particularly those bacteria belonging to the families psendomonaceae, bacillaceae and enterobacteriaceae. Further down the cause of the river, some other species start to appear. These may include *Azotobacter, Vibrio, Spirilium, Thiobacillus, Streptomyces, Nocardia Spirochaetes* and nitrifying bacteria.

Generally, rivers have more fluctuating conditions because of inflowing tributaries and sewage outflows; and bacteria populations are variable depending on local conditions. As for the lakes, they are more selfcontained than streams and rivers and in clean waters, most of the bacteria encountered are Gram negative such as *Pseudomonas, Flavobacterium, Micrococcus, Streptrococcus* etc.

The vertical distribution of bacteria varies seasonally with stratification and nutrient distribution. Bacteria are usually high in sediments where organic materials are abundant. In the marine environment, the bacteria species present are only slightly halophilic as compared with the true halophiles such as *Halobacterium* species. Most bacteria present in this kind of environment are Gram negative rods which are motile and facultative psychrophiles. Their numbers are slightly high where organic nutrients are available particularly near water stored sediments. Some of the common genera are *Micrococcus, Corynebacterium, Spirillum, Norcardia, Streptomyces etc.*

Microalgae: - The aquatic microalgae play an extremely important role in aquatic environments as primary producers of organic compounds. They are basically autotrophic, but a few of them can be heterotrophic. In small bodies of water, such as ponds, much of the primary productivity may be carried out by benthic algae but in large bodies of water e.g. large lakes and oceans, algal phytoplanktons are the main producers. In the fresh water; the most in important phylla are the blue green, yellow-brown algae and diatoms. Whereas in the sea, the most important representatives are the diatoms and the dinoflagellates. The distribution of algae is determined by environmental factors such as temperature and nutrients supply. In fast flowing habitats, microalgae are usually found on stones or living in bottom sediments but in calm water where conditions are favorable, growth of plantonic algae may be very rapids resulting in large accumulation of cells called "algal blooms" e.g. blue-green algal blooms which occurs in neutrophic fresh waters.

Fungi: - All fugal species are heterotrophic. The algae are the producers. Aquatic fungi are important in decompositionbecause they are able to break down fat, cellulose, hemicellulose, liqnin, chitin, proteins and carbohydrates etc in water. Fungi are more important than bacteria in the breakdown of complex materials. su Representatives of four major classes of fungi are found in aquatic environments, either free living or more often growing on surfaces. But strictly speaking, the real aquatic fungi are the primitive phycomycetes such as blastocladiales, chytridiales and saprolegniales. The distribution of members of the saprolegunales is dependent on the presence of plant and animal materials which they parasitize or consume when dead. They are therefore rare in ground water and springs, because of low nutrients contents, but in rivers, the lower fungi are important. While yeasts may be quite common in sewage out flows, in lakes and oceans, phycomycetes may be common particularly those belonging to chytridiales and saprolegniales. Marine fungi are both halophilic and halotolerant.
Many of the phycomycetes are parasite of plants and animals.

UNIT3. MORPHOLOGICAL, PHYSIOLOGICAL AND GENETIC ADAPTATIONS OF MICROORGANISMS TO THEIR ENVIRONMENTS.

Introduction

Aquatic, terrestrial and aerial ecosystems all over the planet support large and diverse microbial populations. In this units, we shall considered the various adaptations of micro-organisms that enable them to survive in these ecosystems.

Objectives

The objective of this units to make the audience understand and comprehend the various ways and mechanisms micro-organisms have developed or evolved either physiologically, morphologically or genetically to be able to withstand extreme conditions in their environments.

Microbial adaptation to Marine and Freshwater Environments. Marine and freshwater environments have varied surface areas and volumes. They are found in location as diverse as the human body, beverages and the usual places one would expect- rivers, lakes and the oceans. They also occur in water- saturated zones in materials we usually describe as soils. These environments can range from alkaline to extremely acidic, with temperatures from -5 to -15^{0}c at the lower range, to at least 121^{0}c in geothermal area. Some of the most intriguing microbes have come from the study of high temperature environments, including the now classic studies of Thomas Brock and his co-workers at Yellowstone National Park. Their work led to the discovery of *Thermus aquaticus,* the source of the temperature-stable DNA polymerase which makes Polymerase Chain Reaction (PCR) possible. In addition to temperature, the penetration of sunlight and the mixing of nutrients, oxygen and waste products that occur in freshwater and marine environments are dominant factors controlling the microbial community. Water provides an environment in which a wide variety of microorganisms survive and function. Microbial diversity depends on available nutrients, their varied concentrations, the transition from oxic to anoxic zone and the mixing of electron donors and acceptors in this dynamic environment. In addition, the penetration of light into many anoxic zones creates environments for certain types of photosynthetic micro-organisms.

One of the adaptation of marine micro-organisms is how small most oceanic microbes are. They are so small that not until the development of very fine filtration systems and the application of direct counting methods (such as epiflourescence microscopy) that the abundance of ultramicrobacteria was discovered. The small size is an adaptation because microbial cells must assimilate all nutrients across their plasma membrane.

Cells with a large surface area relative to their total intercellular volume are able to maximize nutrients uptake and can therefore grow more quickly than their larger neighbours. Thus, the majority of microbes growing in nutrient- limited or oligotrophic open oceans are between 0.3micrometer and 0.6micrometer Such microbes have evolved to maximize their surface area to volume ratio to oligotrophic conditions.

At the other extreme is a usual marine microbe found off the coast of Namibia in West Africa. *Thiomargarita namibiensis,* which means the "Sulphur pearl of Namibia" is considered to be the world's largest bacterium. Individual cells are usually 100-300 micrometer in diameter. Sulfide and nitrate are used by the organism as electron door and acceptor respectively. In this case, nitrate from the overlying seawater, penetrates the anoxic sulfide-containing mud' only during storms. When this short term mixing occurs, *Thiomargarita* takes up and stores the nitrate in a large internal vacuole which occupies 98% of the organisms volume. The vascular nitrate can approach a concentration of 800mili micron. The elementary sulfur granules appear near the cell edge in a thin layer of cytoplasm. Between storms, the organism lives using the stored nitrate as an electron acceptor. These unique bacteria are important in sulfur and nitrogen cycling in these environments.

Another critical adaptation of micro-organisms in aquatic systems is the ability to link and use resources that are in separate locations or that are available at the same location only for short intervals such as during storms. One interesting bacterium linking widely separated resources in *Thioploca* species (the spaghetti bacterium) which lives in bundles surrounded by a common sheath. These microbes are found along the coast of Chile, where oxygen poor, nitrate rich waters are in contact with sulfide – rich bottom mud. A similar situation obtains in the coast of West Africa. Such bacteria form filamentous sheath structures, and the individual cells can slide 5-15cm deep into the sulfide- rich sediments.

Again, Zoosporic organisms adapt to life in the water by having asexual reproductive spores with a single whiplash flagellum e.g. chytrids. Another important group is the filamentatous fungi that sporulate under water. These hyphomycetes include the "Ingoldian fungi" named after C.T Ingold in 1942. The ecology of these aquatic fungi is very interesting. They produce a unique tetraradiate conidium on the vegetative mycelium which grows inside decomposing leaves. When the vegetative hyphae differentiate into an aerial mycelium, conidia are released into the water. The released conidia are then transported and often are present in surface foams. When they contact leaves, the conidia attach and establishe new centers of growth.

It is important to note that about 97% of the Earth's water is in marine environment (estuaries, open oceans and dark cold high pressure benthos) much of which is in the deep sea. The surface waters have been studied more intensely by microbiologist, because this is where the photosynthesis that drive all the marine ecosystems takes place. It is only recently that scientist had the capacity to probe the deep sea sediments and the subsurface (the benthos).

An estuary is a semi enclosed coastal region where a river meets the sea. They are defined by tidal mixing between fresh-water and salt water. They feature a characteristic salinity profile called a "salt wedge". The salt wedges are formed because salt is denser than freshwater, so sea water sinks below the overlying freshwater. As the contribution from the freshwater increases, and that of the ocean declines, the relative amount of seawater declines with the estuary's increased distance from the sea. The distance the salt wedge intrudes up the estuary is not static.

Most estuaries undergo large scale tidal flushings and this forces organisms to adapt to changes in salt concentrations on a daily basis. Microbes living under such conditions combat the resulting osmotic stress by adjusting their intracellular osmolarity to limit the difference with that of the surrounding water. Most protists and fungi produce osmotically active carbohydrates for this purpose; whereas prokaryotic microbes regulate internal concentrations of potasium or special amino acids (ecoine and betaine). Most other microbes that inhabit estuaries are halotolerant, distinct from halophilic. Halotolerant microbes can with-stand significant changes in salinity, halophilic micro-organisms have an absolute requirement for high salt concentrations.

Estuaries are unigue in many respects. Their calm nutrient-rich waters serve as nurseries for juvenile forms of many commercially important fish and invertebrates.

However, they are the most polluted marine environments. They are the ultimate receivers of wastes that are dumped in rivers, and pollutants discharged from industries.