NATIONAL OPEN UNIVERSITY OF NIGERIA

SCHOOL OF SCIENCE AND TECHNOLOGY

COURSE CODE: SLM 505

COURSE TITLE: SOIL MICROBIOLOGY AND BIOCHEMISTRY

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COURSE GUIDE

SLM 505
SOIL MICROBIOLOGY AND BIOCHEMISTRY

NATIONAL OPEN UNIVERSITY OF NIGERIA

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INTRODUCTION

Soil microbiology and Biochemistry is a discipline that fundamentally seeks to study the interactions and activities of soil organisms, especially those in the invisible world of the naked eyes, living in the soil environment. Living organisms, both plant and animals, constitute a crucial component of soil system. Although these organisms form a mere fraction of less than 1 % of the total soil mass, they still play significant role in supporting the plant communities of the Earth. The study of Soil Microbiology and Biochemistry, based on scope and importance, can best be appreciated while considering, amongst others, the following: soil as a living system, soil microorganisms and plant growth, soil microbes and soil structure, organic matter and its decomposition, humus formation, biogeochemical cycling of nutrient elements, soil microbes as bio-control agents, role of soil microbes in seed germination, biological N_2 fixation, degradation of pesticides in soil, *et cetera*.

This course sought to introduce students to the basics of the methods and techniques involved in the isolation and estimation of soil microorganisms. It will also make them to become familiar with some aspects of certain soil microbes vis-à-vis their activities, depending on conditions. Biological nitrogen fixation and biogeochemical cycling, for example, are controlled and executed by some special microorganisms that are naturally available to undertake the processes; whereas, some others like mycorrhizas and rhizosphere microbes do other certain functions. Essentially, we are talking of Soil Microbiology and Biochemistry as a course of study that generally intimates students with the first-hand knowledge on the roles of microorganisms living in soil in ensuring a healthy soil condition for practical and effective management of agricultural crops for an ensured food security and general environmental stability.

WHAT YOU WILL LEARN IN THIS COURSE

This course carries two credit units.

This course guide tells you briefly what to expect from reading this course material. The study of Soil Microbiology and Biochemistry may be described as the cross breeding of two disciplines, soil microbiology and soil biochemistry. The microbiology component deals with the general soil microbial ecology and the biochemistry aspect all biochemical processes taking place in the soil and soil components as well as that occurring within living components of the soil environment. It is these relationships and interactions between the soil environmental biota and the plants and animals, micro and macro, that constitute the major concern of Soil Microbiology and Biochemistry.

COURSE AIM

The course aims at providing a good and first hand understanding of the soil microbial ecology as well as corresponding biochemical processes taking place therewith.

COURSE OBJECTIVES

After going through this course, you should be able to:

- Explain the various roles of soil microorganisms
- Explain the elements and factors of weather and climate
- Explain the dynamics of the atmosphere

- Appreciate the dynamics of pressure and wind systems.
- Appreciate the seasonal variations in the different factors of the climate
- Identify the equipment used to measure the various elements of climate and state how to maintain them
- Expressly state the relationship between climate and agriculture.

WORKING THROUGH THIS COURSE

This course has been carefully put together bearing in mind the fact that it is an introductory course. However, efforts have been made to relatively ensure an adequate explanation of the concepts and issues treated in the work. Tables and Figures, where helpful, have been used in for an enhanced understanding. You are therefore, advised to spend good time to study the work and ensure that you attend tutorial sessions where you can ask questions and compare your knowledge with that of your classmates.

COURSE MATERIALS

You will be provided with the following materials: A Course guide

Study Units.

In addition, the course comes with a list of recommended text books and related reference materials which are not compulsory for you to acquire or read, but are essential to give you more insight into the various topics so discussed.

STUDY UNITS

The course is divided into 15 units. The following are the study units contained in this course:

Module 1

Unit 1	Role of Microorganisms in Soil
Unit 2	Methods of Isolation and Estimation of Soil Microbes
Unit 3	Factors Affecting Abundance of Bacteria in Soil
Unit 4	Rhizosphere Microbes
Unit 5	Nitrogen Cycle

Module 2

Unit 1	Phosphorus Cycle
Unit 2	Biological Nitrogen Fixation
Unit 3	Mycorrhiza
Unit 4	Organic Matter
Unit 5	Fate of Crop Residues

Module 3

Unit 1 Animal Wastes in Soils
Unit 2 Sewage Materials in Soils
Unit 3 Petroleum Hydrocarbons in Soils
Unit 4 Detergents in Soils
Unit 5 Pesticides in Soils

Module 1

In unit one, you will be introduced through the various roles of microorganisms in the soil system. In unit two, the various methods involved in the isolation and estimation of the soil microbes will considered whereas unit three will consider the factors that affect the abundance of soil bacteria in the terrestrial environment. In unit four, rhizosphere microorganisms will be introduced and nitrogen cycle, one of the biogeochemical cycle components will be covered in the unit, five, of the first module.

Module 2

In unit one, you will be taken through phosphorus (P) cycle and P cycle related topics. Unit two will be on biological nitrogen (N) fixation and the nitrogenase complex and the mechanisms involved in its role and function. Unit three is on mychrrhizas while units four and five will take you through organic matter and the fate of crop residues in soils, respectively.

Module 3

In unit one, you will learn about animal wastes. It will discuss the various types and impact on soil and organisms. In unit two, you will be introduced to sewage materials in soils. Their role and importance will also be introduced. The remaining units, three to five will respectively introduce the conditions of soil and soil microbial communities under the influence of petroleum hydrocarbons, detergents and pesticides, respectively. Their effects of interactions with soil biota will be attempted.

TEXT BOOKS AND REFERENCES

The following textbooks are recommended for further reading

Aislabie, J. and Deslippe, J.R. (2013). Soil Microbes and their Contribution to Soil Services. *Soil Microbial Diversity*, 143-161. Lincoln, New Zealand: Manaaki Whenua Press.

Giller, K.E. and Wilson, K.J. (1991). *Nitrogen Fixation in Tropical Cropping Systems*. Wiltshire, UK: C.A.B International.

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Postgate, J. (1978). *Nitrogen Fixation.* London: Edward Arnold (Publishers) Limited.

Sylvia, D.M., Fuhrmaan, J.J., Hartel, P.G. and Zuberer, D.A. (Eds.) (2005). *Principles and Applications of Soil Microbiology* (2nd Edn). New Jersy, USA: Pearson Prentice Hall™ Ltd.

ASSESSMENT

There are two components of assessment for this course. They are the Tutor-Marked Assignment (TMA), and the end of course examination.

TUTOR-MARKED ASSIGNMENT

The TMA is the continuous assessment component of your course. It accounts for 30 % of the total score. The TMAs will be given to you by your facilitator and you will return it after you have done the assignment.

FINAL EXAMINATION AND GRADING

This examination concludes the assessment for the course. It constitutes 70 % of the whole course. You will be informed of the time for the examination.

SUMMARY

This course intends to provide you with the knowledge of the roles of soil microorganisms and some of the biochemical activities they mediate within the soil environment so as to make our agricultural soils healthier for enhanced crop productivity. By the end of this course, you will be able to answer the following questions.

- Discuss on the role of microorganisms in the soil.
- Itemise the methods of isolation and estimation of soil microorganisms and discuss any three of them.
- Differentiate isolation from estimation of soil microbes
- Discuss on the factors that affect the abundance of bacteria in the soil.
- Discuss biogeochemical cycle with an especial reference to nitrogen (N) and phosphorus (P).
- Compare and contrast between the N and P cycles
- What do you understand by rhizosphere microorganisms? Support your answer with clear examples.
- Differentiate rhizosphere from edaphosphere in terms of microbial activities in soils.
- Discuss on the significance of biological nitrogen fixation in agriculture.
- Which of the BNF quantification methods do you think to be the best of all? Reasons?
- Give a full account of your understanding on mycorrhiza.
- Write a detailed note on organic matter.
- Comment freely on the fate of crop residues in the soil.
- Compare, contrast and explain, on the importance of, animal wastes and sewage materials.

- Explain the influence of microorganisms on petroleum hydrocarbons, detergents and pesticides in soil and vice-versa.

We believe you will find the course very interesting and so hope that you will have a better understanding of it. We also wish you success in the course.

Good luck.

MAIN COURSE

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MODULE 1

UNIT 1 THE ROLE OF MICROORGANISMS IN SOIL

CONTENTS

1.0 INTRODUCTION

Microorganisms, also called microbes, are very minute (small) forms of life that can, sometimes, live as single cells, though many also form colonies of cells. Microscope is usually necessary to view individual cells of these organisms. Much more microbes exist in topsoil (0 – 5 cm), where food sources are plenty, than in subsoil (deeper than 5 cm). They are especially even more abundant in the area immediately next to plant roots (called the *rhizosphere*), where sloughed-off cells and chemicals released by roots provide ready food sources. These organisms are the primary decomposers of organic matter, although they also play such other roles, as providing nitrogen (N) through fixation to help growing plants, detoxifying harmful chemicals (toxins), suppressing disease organisms and producing products that might stimulate plant growth. Soil microorganisms have had another direct importance for humans. They are, for example, the source of most of the antibiotic medicines we use to fight diseases.

Microorganisms are most famously known, by most people, for their negative impacts in terms of disease causing agents. But turning towards their positive impacts, they also have an important role on agricultural production by rendering a helping hand to plants' growth. Microorganisms have been an important integral unit of soil from the antiquities of earth formation. They are capable of turning waste land into a productive soil, as they increase soil fertility through the incorporation of air, nitrogenous and other mineral compounds. They are good at contributing to an increased plant growth by making essential elements and other, otherwise plant-unavailable, minerals readily accessible. Microbes decompose organic matter to such simpler forms that can be easily taken up by plants. It is a verified fact that, although soil organisms comprise < 1 % of the total mass of soil, a hand full of soil is composed of millions of microorganisms which assist in boosting soil fertility and, hence, plant growth. The microbial population of a given soil is proportional to its fertility. The number of a given species of microorganism, however, directly depends on physical and chemical properties of the soil. This fact can easily be demystified when one considers that different microbes decompose different organic matter. Microbes are, therefore, a major subject of investigation in the recent past due to the role they play in improving soil fertility.

Many nutrients are developed in soil as a result of biological transformation by the action of microorganisms thereby affecting soil functioning by making it fertile. These eye-invisible beings ensure a, relatively, permanent existence of nutrients in the soil. This ensures a successful agriculture when the nutrients are managed effectively. Nutrient status in soils of agro ecosystems is largely determined by identification and quality of inherent microorganisms. This assists farmers in maintaining these nutrients so as to ensure higher crop yields. Various studies have also shown that microbial community structure is dictated by environmental factors and chemical properties of soil. Soil nutrients may, for

example, be influenced by soil management which results in such processes as erosion, oxidation, leaching and mineralisation. The activity of soil microorganism may either be increased or decreased due to these processes, and which consequently results in affecting the soil fertility.

2.0 OBJECTIVE

At the end of this unit, you should be able to:

- enumerate the various roles of microorganisms in the soil

3.0 MAIN CONTENT

3.1 Microorganisms in the soil

According to findings based on molecular phylogeny, there are generally three domains of living organisms as thus: Bacteria, Archaea and Eucarya (Woese, 1990). This is glaringly different from the traditionally recognised five kingdoms (bacteria, fungi, Protista – that included algae and protozoa), animals and plants).

3.1.1 Bacteria

Bacteria are all procaryotes that are not members of the domain Archaea, and generally live in almost any habitats. They are present inside the digestive system of animals, in the salt and fresh water, in compost piles (even at temperatures over 54 °C) and in soils. Although some types of bacteria live in anaerobic conditions in flooded soils, most of them require well aerated soil environments. Bacteria tend to thrive better in soils with a neutral pH than in acidic ones. Besides being among the first organisms to initiate decomposition process of residues in the soil, bacteria additionally benefit plants by promoting nutrient availability. Many bacteria, for example, dissolve phosphorus (P), thereby making it more available for plants' uptake. Bacteria are also important in the provision of nitrogen (N) to plants, which they necessarily need in large quantities but being most often deficient in many agricultural soils. One may wonder how soils can be N-deficient when 78 % of the air for breathe around us is occupied by nitrogen gas (N_2). Yet plants, as well as animals, face the "Ancient Mariner" dilemma, who adrift at sea without fresh water: "Water, water, everywhere no any drop to drink." Neither animals nor plants, unfortunately, can use N_2 for their nutrition.

Some bacteria types are, nevertheless, capable of taking N_2 from the atmosphere and converting same into a plant-utilisable form from which plants make amino acids and proteins. This conversion process is known as biological nitrogen fixation (see Module 2), in which some nitrogen-fixing bacteria form mutually beneficial associations with plants. People eat some legumes and/or their products. Groundnut and cowpea haulms, soya beans, alfalfa and clover are, amongst others, used for animal feed. Clovers, mucuna, centrocema *et cetera* are grown as cover crops to enrich the soil with organic matter, as well as N source, for the following crop.

Another group of bacteria is *Actinomycetes*, which break large lignin molecules (large and complex molecules found in plant tissue, especially stems, that is so difficult for most organisms to break down into smaller sizes). Lignin also intermittently protects other molecules, like cellulose, from decomposition. *Actinomycetes* have some features similar to those of fungi, but are occasionally grouped by themselves and given equal standing with bacteria and fungi.

3.1.2 Archaea

This is an evolutionarily distinct domain (*i.e.* group) of procaryotes that consists of *methanogens*[1], most extreme *halophiles*[2] and *hyperthermophiles*[3] and *Thermoplasma*. The distribution of archaeal phyla in the soil has been the subject of numerous 16S rRNA gene surveys. These surveys have revealed archaeal widespread presence, primarily members of the *Crenarchaeota* phylum, in soil. They are mostly more abundant below the topsoil. Although *crenarchaea* are relatively diverse, the ones abundant in soils tend to be restricted to one specific lineage, the group 1.1b. Evidence indicated soil *crenarchaea* as contributing to ammonia (NH_3) oxidation in soil. Studies on soil metagenomics have also revealed *crenarchaea* that is affiliated to lineage group 1.1b as containing and expressing *amoA* genes. Recently (Tourna et al. 2011), an NH_3-oxidising *crenarchaea*, identified as *Nitrososphaera viennensis*, was isolated from garden soil, and subsequent phylogenetic analysis confirmed its taxonomic affiliation with the group 1.1.b.

Euryarchaeota, specifically methanogens, are present in soil but active only in anoxic conditions, for example under waterlogged soil conditions. They are, therefore, strict anaerobes and grow in association with bacteria where they partake in the anaerobic food chain and convert complex organic molecules into methane (NH_4) and CO_2. The pathways that methanogens use to generate CH_4 vary. They include CO_2 reduction and methanol (CH_3OH), cleavage of acetate, and production of CH_4 from methylated compounds. In soil, methanogens belonging to the genera *Methanosarcina, Methanosaeta*, and *Methanocella* are widespread. Both *Methanosarcina* and *Methanosaeta* reduce acetate to produce CH_4.

3.1.3 Eucarya

Eucarya or Eucaryota (also spelled Eukarya), is derived from the Greek *eu* meaning "well" or "true", and *caryon* meaning, "nut" or "kernel". It is a domain of organisms that have cells, each, with a noticeable nucleus within which is their genetic material. Eucaryotic cells also have other such membrane-bound organelles as mitochondria and the Golgi apparatus in addition some plants and algae cells that contain chloroplasts. Like the *Bacteria*, Eucarya also have membranes that are composed of un-branched fatty acid chains attached to glycerol by ester linkages as indicated in Figure 1. Unlike unicellular Archaea and bacteria, eucaryotes may also be multicellular and include organisms consisting of many cell types that form different kinds of tissue. Plants and animals are the most known eucaryotes; however, there are four kingdoms in the Eucaryotic domain, as thus: animalia, plantae, fungi and protista (unicellular organisms).
All animals are eukaryotic. *Animalia* cells are noticeable from those of other eukaryotes', most notably plants, as they lack cell walls and chloroplasts and have smaller vacuoles. Due to the lack of a cell wall, animal cells can transform into a variety of shapes. A phagocytic cell is even capable of engulfing other structures. Plantae are multicellular organisms that are composed of eucaryotic cells. The cells are organised into tissues and have cell walls. They obtain nutrients photosynthetically and by absorption. Examples include flowering plants, ferns, mosses and conifers. Fungi can be unicellular or multicellular organisms also possessing eucaryotic type of cells. They have cell walls but that are not organised into tissues. Hence, they do not undertake photosynthesis and so obtain nutrients by absorption. Moulds, yeasts, sac fungi and club fungi are a few examples of fungi. Protista are simple and predominantly unicellular eucaryotes that are exemplified by euglenoids, slime moulds, algae and protozoans.

[1] Methane-producing procaryotes
[2] Organisms requiring or tolerating a saline environment
[3] Organisms with growth optimum temperature requirement of > 80 ^{0}C, thermophiles require a range between 45 – 85 ^{0}C as growth optimum temperature requirement

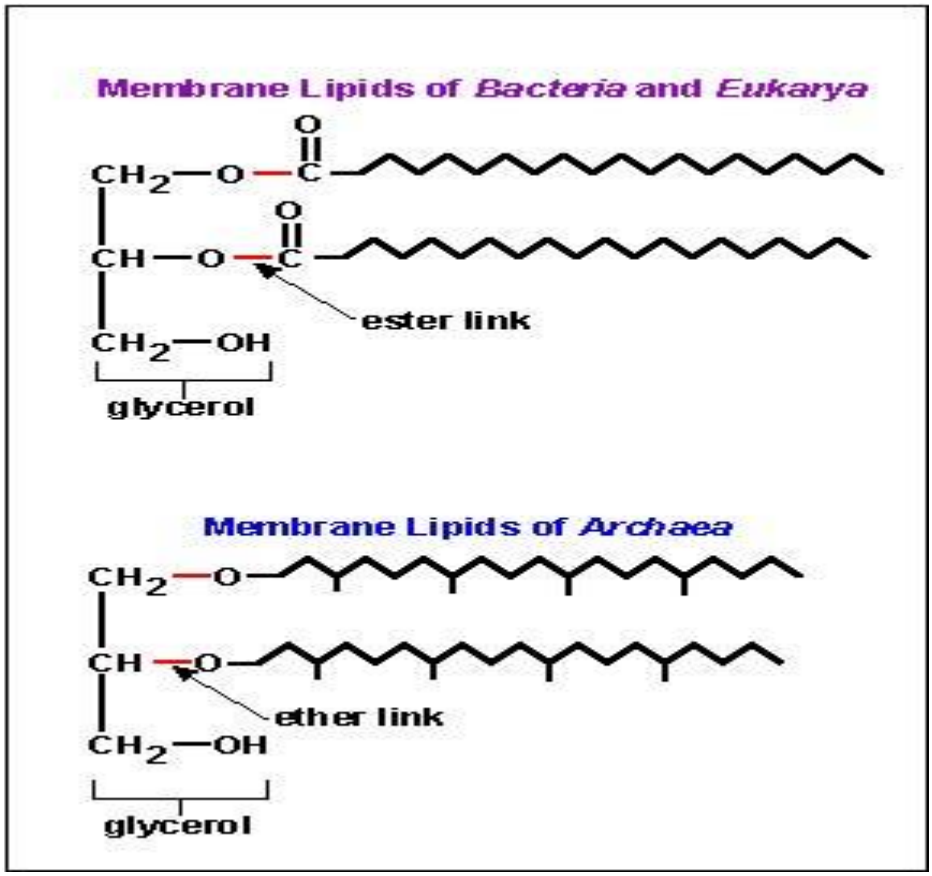

Figure 1 showing membranes of *Bacteria* and *Eucarya* that composed of un-branched fatty acid chains attached to glycerol by ester linkages. Membranes of *Archaea* are composed of branched hydrocarbon chains also attached to glycerol but by ether linkages. Source: Kaiser, G.E. (2018).

3.2 The role of microorganisms in the soil

Microbes in soils play a central role in in diverse ways, including cycling of nutrients that are essential for life, conversion of organic materials that are formed by primary producers back to CO_2 during respiration. The microbes are, sometimes, assisted by such higher animals as herbivores and carnivores by digesting crude organic material with the aid of intestinal tracts microbes (decomposition). Microbes are also important in mineralisation of organic compounds which occurs after being completely degraded completely into such inorganic products as CO_2, NH_3 and H_2O. Fungi are the major agents of organic matter decomposition in the soil ecosystems. Both the fungi and bacteria, however, degrade complex organic molecules impossible for higher organisms to break down. A vast array of bacteria, notably those in Actinobacteria and Proteobacteria, break such soluble organic molecules as amino acids, organic acids and sugars as reported by Eilers *et al.* (2010). Also, some of such bacteria as Bacteroidetes, assist in degrading more resistant C compounds like lignin, chitin and cellulose. Bacteria targeting such recalcitrant C compounds may need relatively high available N rates in order to support extracellular and transport enzymes production (Treseder *et al*., 2011). Low N environments adapting bacteria, in contrast, are more capable of metabolising such organic N compounds as amino acids.

Net C mineralisation was reported as positively correlating, in soils, with the abundance of β-Proteobacteria and Bacteroidetes, and negatively with Acidobacteria's (Fierer et al. 2007). Microorganisms are uncommon in their anaerobic ability to degrade organic matter, resulting fermentation of organic compounds to organic acids, thereby generating such gases as H_2 and CO_2. The H_2, under strict anaerobic conditions, may be utilised by methanogens to reduce CO_2 into methane gas (CH_4). Some methanogens can metabolise methanol (CH_3OH), acetate (CH_3COOH) or methylamine (CH_3NH_2) to CH_4 and CO_2. Table 1 summarises the role of microorganisms in soil.

4.0 CONCLUSION

Soil is an abode for a large population of archaea, microscopic fungi, algae, cyanobacteria, actinomycetes, protozoa, nematodes and macroscopic earthworms and insects. In it, microorganisms play critical roles in organic matter formation, recycling of nutrients, secretion of gums, polysaccharides and glycoproteins and in converting atmospheric nitrogen (N_2) to ammonia (NH_3).

5.0 SUMMARY

In this unit, we have learnt that:

1. There are three domains of living organisms (Bacteria, Archaea and Eucarya) in the soil;
2. The microorganisms comprise < 1 % of the overall soil mass;
3. A hand full of soil contains millions of microorganisms, which assist in booming soil fertility and hence, plant growth;
4. The higher the microbial population in soil the higher its fertility;
5. Microbes have many other vital roles in supporting plant and animals' lives;
6. The population of a given species of microbes depends on soil physical and chemical properties.

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe the general role of microorganisms in soils giving unambiguous examples.
2. Compare and contrast between Eucarya and Bacteria.
3. With clear examples, distinguish phylogenetic from traditional method of classification.
4. Soil health is microbial population dependent. Discuss this assertion with cogent reasons.

7.0 REFERENCES/FURTHER READING

Aislabie, J. and Deslippe, J.R. (2013). Soil Microbes and their Contribution to Soil Services. *Soil Microbial Diversity*, 143-161. Lincoln, New Zealand: Manaaki Whenua Press.

Kaiser, G.E. (2018). Microbiology E-Text and Lecture Guide. http://faculty.ccbcmd.edu/~gkaiser/index.html

Sylvia, D.M., Fuhrmaan, J.J., Hartel, P.G. and Zuberer, D.A. (Eds.) (2005). *Principles and Applications of Soil Microbiology* (2nd Edn). New Jersy, USA: Pearson Prentice Hall™ Ltd.

Table 1 Role of soil microorganisms in provisioning and regulating services provided by soil ecosystems

Soil service	Descriptor	Role of soil microbes
Provisioning services – products obtained from ecosystems		
Physical support	Surface soils of the earth represent the physical base on which humans, animals and infrastructures stand. Soils also give support to animal species (e.g. livestock) that benefit humans.	Microbes contribute to soil formation via nutrient cycling and production of organic matter. Microbial products are crucial to soil aggregation and improved soil structure thereby making soil more habitable for plants.
Raw materials	Soils can be a source of raw materials (e.g. clay for potting and peat for fuel).	Soil microorganisms produce antimicrobial agents and enzymes that are used for biotechnological purposes.
Plants' growth medium	Mankind uses plants for food, fibre, medicines, energy, building and more. Soils, therefore, provide services to humans by enabling plants to grow. Soils provide physical support to plants and supply nutrients and water for them.	Soil microorganisms mobilise nutrients, from otherwise, insoluble minerals to support the plant growth.
Regulating services – enable humankinds to live in a healthy, stable and resilient environment		
Buffering water flows	Soils are capable of storing and retaining quantities of water and can, therefore, mitigate and lessen the impacts of extreme climatic issues (*e.g.* limit flooding). Such soil macroporosity and hydrological processes as infiltration and drainage impact on this service.	Soil macropores, formed by plant roots, earthworms and other soil biota, may depend on soil microorganisms as food or for nutrients.
Nutrient cycling	Soil is the decomposition site for organic materials and the mobilisation of nutrients in bedrock and soil aggregates. Soil is also an oxidation and reduction (redox) site for nutrient elements, symbiotic N_2-fixation and photoautotrophic activities.	The activities of soil microbes (bacteria, archaea and fungi) drive nutrient cycling in soils and are also involved in weathering minerals.
Recycling of wastes and detoxification	Soils absorb, detoxify, and recycle applied wastes (e.g. effluent disposal), agrochemicals, and spills of fuels and oils, reducing potential harm to humans and to organisms useful to humans.	Microbial processes like mineralisation and immobilisation are responsible for this service. Detoxifying microbes may be limited by the availability of soil nutrients (e.g. N or P), which in turn depends on soil microbial activities.
Filtering of contaminants	If pollutants, such as excess nutrients, exotic microbes, metals and organic compounds, are leached from soils, they can readily contaminate aquatic ecosystems and threaten human health. Soils absorb and retain such solutes and pollutants, thereby avoiding their release into water.	In concert with the clay and organic matter contents of soils, microbial products contribute to both the hydrophobicity and wettability of soils thereby impacting on the ability of soils to filter contaminants.
Habitat for biodiversity	A very huge component of global biodiversity occurs in soils. Some organisms have above-ground life stages or are food resources for above-ground species. Soils are therefore a reservoir for resting phases of organisms (e.g. seeds, fungal spores) and hence are critical for the rejuvenation of communities.	Soil microbes (specifically bacteria, archaea, and fungi) comprise the large majority of the biological diversity on earth. They are also the foundation of soil food webs and so underpinning the diversity of higher trophic levels. Interactions among soil microorganisms and plants usually determine the plant biodiversity.
Biological control of pests, weeds and pathogens	Soils provide a conducive habitat for beneficial species that regulate the composition of communities and therefore prevent the proliferation of herbivores and pathogens. This service depends on soil properties and the biological processes driving inter- and intra-specific interactions, including symbiosis, competition and host–prey associations).	Beneficial species include bacteria, archaea, and fungi that support plant growth through increasing nutrient availability and by outcompeting invading pathogens.
Carbon storage and regulation of greenhouse gas emissions	Soils play a critical role in regulating many atmospheric constituents, impacting on quality of air and on global and regional climate. Soils store C as a stable organic matter offsetting CO_2 emissions and are home to microorganisms that emit nitrous oxide (N_2O) and methane (CH_4).	By mineralising soil C and nutrients, microorganisms are major determinants of the C storage capacity of soils. Denitrifying bacteria, fungi and methane producing and consuming bacteria regulate nitrous oxide (N_2O) and methane (CH_4) emissions from soils.

Adapted from Dominati *et al.* (2010) In: Aislabie and Deslippe (2013).

UNIT 2 METHODS OF ISOLATION OF SOIL MICROBES

CONTENTS

1.0 INTRODUCTION

Soil is a reservoir for diverse antibiotic-producing, C- and N-cycle microbes and plants' and animals' microbial pathogens. So also, myriads of other lower plants and animals playing roles in the economy of soil. The ability of detecting and, where possible, isolating these organisms in a pure or mixed culture has increasingly become important to soil microbiologists. The available techniques for detection, and possible isolation, are as a matter of necessity, very selective. This is particularly advantageous when interested in particular already characterised *taxa* and disadvantageous in dealing with either anonymous entities or in broad qualitative studies.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- know the various methods of detecting the presence of microorganisms in soils.
- understand the various techniques used in isolating detected soil microbes.

3.0 MAIN CONTENT

3.1 Sampling problems

Sampling soil microbiological samples involves a lot of problems, usually due to the complexity of the medium being sampled. If the method being used, for example, requires a "generalised" soil sample, then such questions as determining what soil horizons to sample, how many samples to take for variability estimation, where exactly to take the samples to determine spatial variation, how frequent to take the samples to determine temporal variation and what sample size should be used, become a big problem. The entirety of the aforementioned are usually interconnected problems. For example, a larger sample, divided into smaller subsamples after mixing, will differ from many small independent ones (samples). The first will, be expected to, show an experimental or a procedural error, while the second will,

expected to, show a combined procedural error and natural field variation.

3.2 Methods used in microbial ecology

The following methods are employed in the detection and isolation of soil microbial ecology, as thus:

1. Growth habit, form and pattern arrangement detection in soil
2. Specific groups or entire microbiota of microorganisms' isolation
3. Detecting and measuring microbial activities in soil
4. Biomass measurement

3.2.1 Growth habit, form and pattern arrangement detection in soil

Microorganisms in soil can have diverse growth patterns. Some bacteria, for example, form microcolonies on surfaces of mineral or organic particles, some others form colonial structures and others exist as single cells. Some fungi and *Actinomycetes*, on another hand, form visible and distinct diffuse colony forms over many soil particles. These different morphologies may be at different scales, some fungal colony structures can extend to kilometres, while some bacterial colonies can exist on a particle of clay. Additionally, individual cells in one of such structures as these can differ in morphology, depending on the growth cycle stage, physical conditions, nutrient status, *et cetera*. *Arthrobacter* species usually display polymorphism, as their cell shape changes on the basis of its growth rate and age, ranging from coccoid to bacilli cells. It is, more often than not, necessary to relate microorganisms with other objects or structures in soil.

Roots of plant, mineral grains, fungal mycelium, organic materials and arthropods are all colonised by particular types of bacteria and *Actinomycetes*. Many kinds of microbial activities are usually only possible under certain conditions. Most often, the cells distribution in soil reflects their activities on specific substrates or their responses to physical and/or chemical conditions. Some anaerobic cells such as *Clostridium*, for example, will only be present in regions that are or have been anaerobic. Resistant spores' formation is a common response to adverse conditions and can be used to indicate past growth arrangements or habits.

Use of standard techniques is often not possible in order to both locate spatially and identify a microorganism in a given soil sample (you may however, refer to fluorescent antibody methods below). To identify microorganisms, therefore, the cells are, in most cases, first cultured in some way so as to permit for colony formation for subsequent identification. The techniques for detecting form, pattern and arrangement of microbes in the soil can be broken down into:

3.2.1.1 Microscopic methods

The microscopic methods include:

a) Light microscopy
b) Electron microscopy

a) Light microscopy

Many old methods of using direct microscopy to examine soil samples are still used today due to their simple nature. They are still useful especially when smaller soil samples, such as pieces of organic materials or mineral grains, are to be examined.

There are basically two main methods used in visualising the microorganisms in these samples. They are classical stains such as phenol aniline blue; and fluorescent stains such as fluorescein isothiocyanate. Classical stains can be examined after staining with any bright-field, white light microscope, assuming that light can be transmitted through the object under examination. Fluorescent stains uses a stain that emits light at a visible wavelength when illuminated with a far-violet or ultraviolet light. This can be an incident illumination that does not have to pass through the object (see Figure 2).

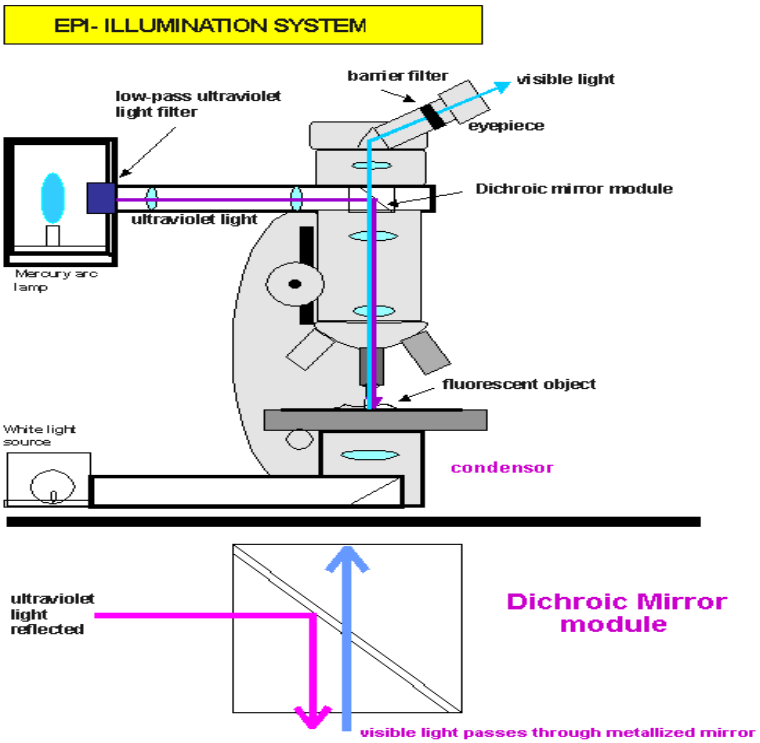

Figure 2. Light microscope showing its illumination system. Source: Kaiser (2018).

The most common fluorescent stains are acridine orange, fluorescein isothiocyanate (FITC) and rhodamine (fluoresces red). Both stains react with parts of the protein molecules, called the sulfhydryl groups, strongly attached to the protein molecules. Other examples are calcofluor, europium chelate, ethidium bromide, Hoechst 33258 (bisbenzimide) and fluorescent probes. Typical examples, of these, are seen in DANSYL chloride and the 8-anilino-1-naphthalene sulphonic acid salts (Mg-ANS and Na-ANS) (Figure 3 and Figure 4). Their major advantage is that they can be applied to soil samples and immediately examined without, necessarily, removing excess unreacted stain. The FITC and rhodamine need an extensive wash up in order to remove unreacted stain.

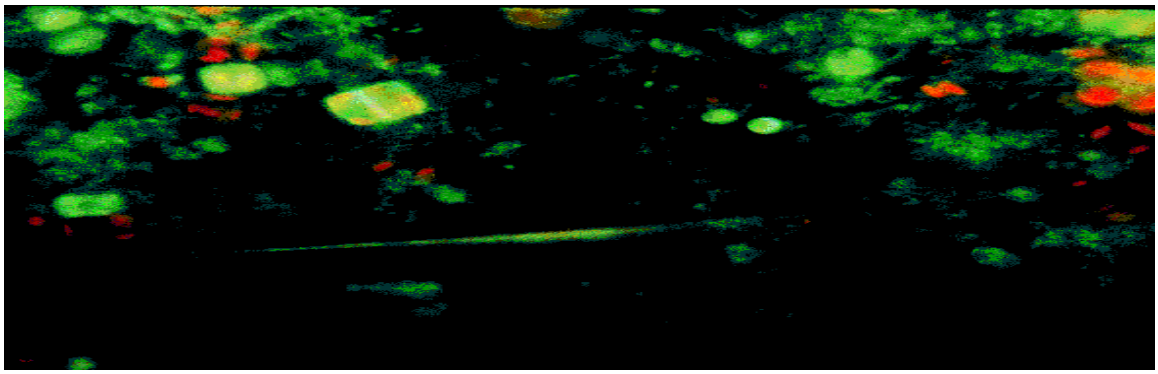

Figure 3 Lake Ontario water stained with Mg-ANS fluorescent probe. The red fluorescence indicates chlorophyll in algal cells and the green fluorescence is cellulose and bacterial cells. Source: Kaiser (2018).

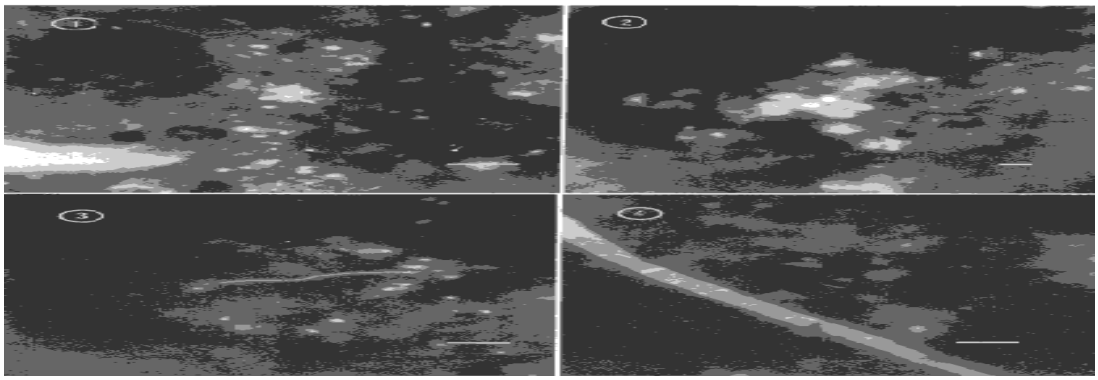

Figure 4 Soil stained with Mg-ANS to show bacterial colonies and fungal mycelium. Source: Kaiser (2018).

b) Electron microscopy

Electron microscopy can also be used in a manner similar to the light microscopy in examining very small areas within soils. Both scanning and transmission electron microscopy have been used, the main disadvantage of both is, however, the effort required to examine even a relatively small volumes of soil. Another hitch is the fixation and coating and/or mounting processes involved. They usually destroy some features of the microbial cells. The process is also characterised by tedium and can, therefore, only examine very small regions of a soil sample.

3.2.1.2 Microscopic methods plus culturing

Soil samples can be saturated with agar or polyacrylate resins, sectioned into thin "plates" soil and examined by direct microscopy. Diamond microtomes are needed to section through mineral grains but the very thin sections resulted can be observed by the use of transmission light microscope. Under the microscopic methods plus culturing, we have the following techniques:

a. Direct culture methods

Culture is a process of growing a biological entity in an artificial medium. Therefore, if soil can completely be removed, placed on a nutrient medium and incubated, then the resulting small colonies can, macroscopically or microscopically, be examined. Using selective media, particular types of microorganisms can selectively be cultured and identified. One technique uses Scotch tape to take successive samples from exposed soil surface. The samples are then transferred into a selective agar medium for incubation. The colonies' position on the plate reveals the original cells' location and distribution in the soil. This technique can, therefore, be combined with replica plating of agar plates to test the colonies' reactions to different media, and thus aiding their identification.

b. Fluorescent antibody and related methods

The fluorescent antibody technique, also known as immunofluorescence method, is the only method that can, concurrently, locate and identify microorganisms in intact soil samples or sections. The microbial cells antibodies are produced by injecting the cells being studied into a suitable animal host, usually guinea pigs or rabbits that are commonly used. After this incubation, the animal used produces antibodies to the microbial cells that can later be isolated from the animal's serum samples. The antibodies, that are proteins, can then be reacted with FITC to produce FITC-antibody conjugates. If applied to a soil sample, the FITC-antibodies will only attach themselves to compatible microbial cells. When excess of these conjugate (FITC-antibody) has been washed out, however, only those microbial

cells will fluoresce and they can be concurrently located and identified by epifluorescence microscopy, just as in FITC-staining.

This method has extensively been used in soil microbiology to identify N_2-fixing *Rhizobium spp*., *Bacillus* spp., many fungal genera such as *Aspergillus* and a few *Actinomycetes*. It has also been used in medical microbiology to identify *Escherichia coli, Neisseria gonorrhoeae, Salmonella* spp., beta-haemolytic streptococci and viruses in tissue samples. One major problem is the relative non-specific nature of many antibody preparations. Many bacteria in same general taxonomic group have similar chemical structures on their cells and so produce an array of antibodies from the structures that overlap with the complex produced by other similar bacteria. Thus, if the antibody complex is used in the formation of the conjugate with FITC the FITC-Ab will cross-react with the other bacteria and target species. This reaction will usually be weak, although still significant. One way of "purifying" the complex is by removing the cross-reacting antibodies via reacting them with the actual cells of bacteria from the unwanted cross-reacting species. Any cross-reacting group of common antibodies will be adsorbed onto the surfaces of added cells and removed from the complex. The remaining antibodies resulted will then be much more specific to the target species.

A more recent modification of this method, however, uses monoclonal or polyclonal antibodies that are produced in other microbial cells to obtain greater amounts of antibodies for conjugation with FITC. Many of these antibodies are now commercially available from suppliers and some are available in already conjugated forms and therefore labelled with FITC and/or rhodamine.

c. Enzyme-linked immunosorbent assays

Enzyme-linked Immunosorbent Assays (ELISA) has also found some application in soils, especially when the population in consideration exceeds 10,000 cells per ml. The technique has been applied mainly to *Rhizobia* in roots of legumes and soil. The major constraint is removal of the microbial cells from the substrates; both direct disintegration (lysis) *in situ* and removal of cells followed by the lysis have been used.

d. Gene probe and nucleic acid hybridization

These techniques rely on detection of specific sequences of nucleic acids in the organism(s) of interest. This method can find specific organisms in soils and other environmental samples if the sequences used are chosen carefully as to be diagnostic. The gene probe is a short segment of nucleotides that specifically binds with the homologous sequence in target microorganism. If the segment is, for example, labelled with radioactive ^{32}P, any binding to the target nucleotides can be detected by the presence of the radioactivity after reaction.

e. The polymerase chain reaction

The polymerase chain reaction (PCR) has recently also been applied to soil microbial ecology. In PCR technique, an extracted deoxyribonucleic acid (DNA) is deliquesced to form single strands, annealed with primers and the DNA extended from the primers by nucleotide addition using a DNA polymerase enzyme. The primers are chosen to link to regions of DNA of interest, near a diagnostic target sequence.

f. Bioluminescence marker genes

Bioluminescence marker genes, typically the *lux* gene of *Vibrio fischeri*, causes photoluminescence in bacterium (*i.e.* emits light). If the gene can be inserted into the target organisms, they become photoluminescent which property can be used in detecting them and tracking their fate in water and soil samples. This same technique has been utilised with *E. coli* and *Pseuodomonas* targets. It has been extended by fusing other genes with the lux gene and inserting both into cells. Naphthalene degradation is promoted by a gene called *nah,* which has been combined with the *lux* gene to make a diagnostic pair. This can footmark both bacteria and their activity in soil samples.

g. Miscellaneous methods

Other various techniques have been employed by individual investigators to determine the microbial distribution in soils. Rossi-Cholodny slides are simple microscope slides that are buried and left in soil for some time before the microscopic examination commenced. Use of strips of transparent chitin and cellulose materials has also been employed. Pedoscopes, on another hand, are small-bored glass capillary tubes that are used in a similar manner.

A few researchers have used direct micromanipulation of soil under a microscope to remove and incubate individual pieces of soil or even individual microbes. It was shown that *Basidiomycete* fungi were much more common using that technique than previously thought. They are under-represented using dilution plates due to rapid growth of other fungal spores. When pieces of fungal mycelium were directly isolated using micromanipulation tools, the *Basidiomycetes* were much more numerous (see Figure 5).

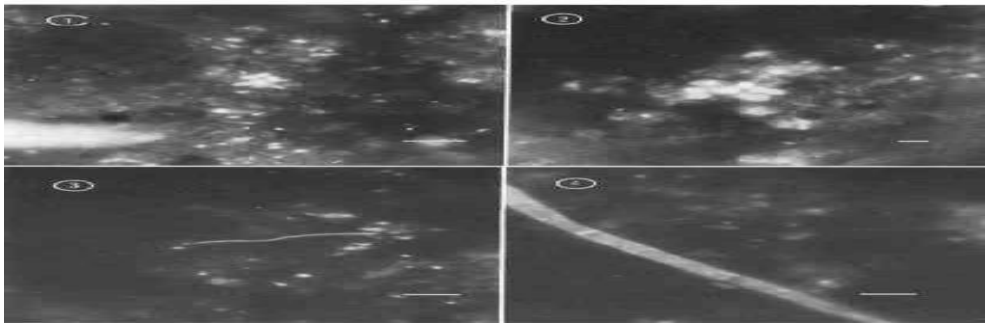

Figure 5 Basidiomycetes population after the use of micromanipulation tools. Source: Kaiser (2018).

3.2.2 Specific groups or entire microbiota of microorganisms' isolation

a) Elective culture methods

If specific chemical compounds are added to a soil in the field or in the laboratory and incubated under given conditions, the organisms that are capable of growing under those conditions will proliferate and come to comprise a greater percentage of the microbiota total. Alternatively, when inhibitory chemicals or incubation conditions are specifically used, specific parts of the microbiota will be decreased in the overall percentage. This simple concept is the basis for the large fraction of industrial uses of soil microbiology, including isolation of hydrocarbon-degrading bacteria, pollutants (*e.g.* pesticides, PCBs, organochlorines, *etc.*) degrading-bacteria, other bacteria (including *Actinomycetes*) or antibiotics-producing fungi and several other examples. This technique can usually be employed to isolate

microbes of any desired property. To isolate a bacterial isolate capable of degrading cellulose under acidic anaerobic conditions, for example, it is only necessary to add cellulose, in form of paper, cotton, *etc.*, to an acetic acid or sulphur acidified-soil sample that is anaerobically incubated.

a) A more sophisticated approach may be finding a soil that has naturally been subjected to conditions that are similar to those that one wish to use as a sample source. Thence, a peat bog would have a microbial population that has already adapted to acidic and anaerobic conditions with a presence of cellulose. Using that as a source of material may probably lead to highly successful isolations.

In a similar vein, soil from an oil refinery contains more hydrocarbon degrading bacteria than farm field soils. In spite of this however, it is still common to find soils containing a large species of organisms seemed not related to that soils. Obviously therefore, many farm field soils also contain hydrocarbon degrading bacteria. This could possibly be a reflection of the great degree of diversity in soil that lead to diverse microbial niches, as a result of biochemical diversity even within an individual species of microbes. Many bacteria can, for example, degrade a wide array of compounds. Elective culture methods can be in one of the two forms below:

i. Selective culture and media
ii. Non-selective media and methods

i. Selective culture and media

All the media used in microbiological laboratories are selective to a certain degree. There are virtually no truly non-selective media. It is possible to make media and incubation conditions very selective by physical and chemical modifications. This is usually very useful in isolating and counting a particular bacterial or other organisms' group(s) from soil samples. There exist routine ways of selective media production, including as follows:

a). Addition of compounds that is used by an organism as a source of nutrient.

b). Omission of compounds required by most other organisms, for example omitting nitrates and other fixed N sources in isolating N_2-fixing bacteria.

c). Addition of such selectively biocidal compounds as penicillin to inhibit gram-positive bacteria, neomycin and streptomycin as general bacterial inhibitors; and actidone and nystatin as general fungal inhibitors.

d). Changing such chemical properties as pH, redox potential (pE), *et cetera*.

e). Altering such incubation conditions as light, temperature, osmotic pressure, water content, *et cetera*.

Using the combinations of these techniques, it is very possible to design very selective media. An isolation medium for *Pseudomonas* is, for example, very specific for its named bacterial group. In theory, almost any physiological group of organisms can be selectively cultured. Single-stage isolation from a soil sample can be changed to a multistage isolation process by replica plating. Individual colonies can be transferred in their original orientation on the plate by pressing a pad of sterile velvet cloth over the plate surface and removing a small sample of each colony and pressing same over a fresh plate surface. This can, however, be a different growth medium so that only a part of the original population is capable of growing on the new medium. In this way, progressive selective media can readily be used in isolating bacteria with combinations of properties. To promote detection of a

particular group of microbes under study, it is also possible to improve the diagnostic precision of the media by using some properties of the organisms like their pigmentation, fluorescence under ultraviolet, biochemical reactions with extra, added substrates, *et cetera*).

ii. Non-selective media and methods

The so-called "non-selective" media are only conditions of media and incubation so designed to isolate as large part of the microbiota in soil as possible. Truly, again, non-selective media do not exist. The least selective media today may isolate 1 to 10 % of the total soil bacteria and may be 5 to 15 % of the fungal population of soils. The media used for bacteria and fungi are different.

3.2.3 Detecting and measuring microbial activities in soil

3.2.3.1 General activity measurements

a) Respiration measurements (O_2 uptake or CO_2 evolution)
b) Rate of cell division
c) Mycelial extension
d) Enzyme activity or content
e) Rate of substrate utilisation
f) Rate of product accumulation
g) Radioisotope cycling studies

a) Respiration measurements

Respiratory activity, as a general measurement of activity, is well established. The Biometer flask is a common apparatus (Figure 6) that is used in measuring the soil samples' activity. The soil is incubated at a given required temperature, moisture content, *et cetera* in the main flask and air can be introduced via the CO_2 absorber in the tube on top of the main flask. During incubation, the CO_2 so produced through respiration is absorbed into an alkali in the side tube. This is then titrated to estimate the quantity of the alkali that is neutralised by the evolved CO_2. More air can be added and the alkali replaced to continue the analysis process. To obtain reproducible results from assays of respiration rate, the soils will usually have to be sieved to remove plant, insects and other related materials. This destroys the aggregate structure and may, therefore, stimulate activity due to release of nutrients that initially protected the inner side of the aggregates. Any kind of disturbance usually causes a temporary burst in respiratory activity.

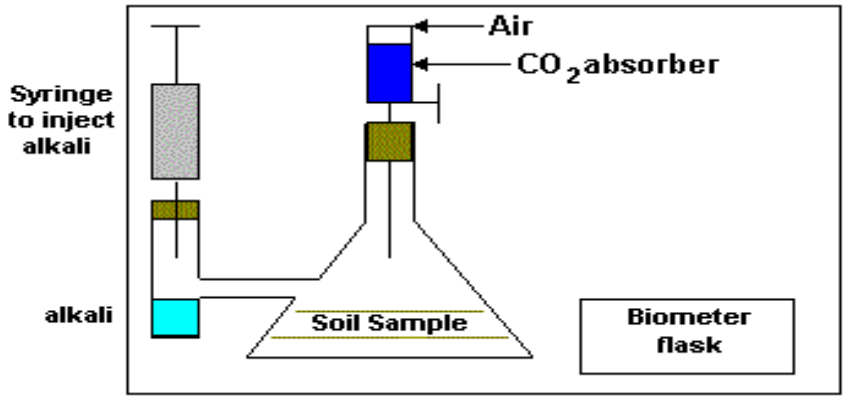

Figure 6. Measuring microbial respiration using biometer flask. Source: Kaiser (2018).

b) Rate of cell division

Rates of cell division can be estimated by a number of counting methods, including direct observation or plate counts, during a given period. If *in situ* rates are required, the very slow growth rate of soil microbes makes it a really difficult measurement to be obtained. Autoradiographic tracer experiments can be used in tracing rates of cell division.

Nalidixic acid inhibits cell separation after cell growth, so that application of this antibiotic to samples will lead to long filamentous (growth of) cells of bacteria. Such vital stains as ANS or Calcofluor can be used in tracing microbial cell division or a mycelial extension of fungi or *Actinomycetes*.

c) Mycelial extension

Hence, fungi grow by the extension of mycelium. If this can be observed, then it can be used to estimate growth rates. Direct observation or extraction from soil medium, followed by observation and measurement, can thus be employed.

d) Enzyme activity or content

Dehydrogenase enzymes can be extracted from soil samples, reacted with tetrazolium dyes to form coloured products and the colour then measured by colorimertic methods. This gives an estimated dehydrogenase activity in the soil sampled and thus an estimate of organisms therein. Various sieving methods can be employed in separating small soil animals, fungi and bacteria from the soil samples, but all are somewhat unsatisfactory, as they do not completely separate the organisms even in a well-mixed soil suspension. Other enzymes that have been used in estimating activities include oxido-reductases, transferases, phosphatases, proteases and cellulases.

e) Rate of substrate utilisation

Any compound that is a substrate for microbes can be utilised in estimating their activity if the substrate concentration's decrease can be measured. Examples are: carbohydrates, detected and measured by the anthrone reaction; amino acids, detected and measured by the ninhydrin reaction; proteins, detected and measured by the Biuret reaction (Folin), and so on. There are always problems involving interference with assays, problems of extraction and change in conditions as the substrates are used by the microorganisms, amongst others.

f) Rate of product accumulation

Any detectable microbial activity product can be used. Transitory products are, however, difficult to assay. There are problems with measurements of both substrate utilisation and product accumulation rates. This due to the fact that:

1. Disappearance of substrates and/or accumulation of products, pH, Eh, CO_2 and O_2 levels, *et cetera* conditions change.
2. Metabolites may, sequentially, be used and removed at different rates, depending on prevailing conditions.

g) Radioisotope cycling studies

Many elements can be obtained in radioactive isotope (^{14}C, ^{32}P, ^{15}N, ^{3}H (tritium), *et cetera*) form. They can be used to measure activities of microbes that are growing and metabolizing compounds that include these isotopes. For example, glucose can be labelled with ^{14}C in specific positions in the glucose molecule and the fate of the ^{14}C can then be traced through standard chemical analysis following radioactivity detection in specific fractions. If the ^{14}C has, however, been evolved as CO_2 through respiratory cells, it will make the CO_2 radioactive, which can be detected. The use of autoradiography can, alternatively, be employed.

h) Autoradiography

In a situation where a uniformly labelled ^{14}C-glucose is, for example, added to a soil and incubated, the rate of ^{14}C uptake into cells can be detected using the autoradiography technique. The cells, in this technique, are being added in suspension to the surface of a photographic emulsion on a plate. During incubation, in darkness, the radioactivity in the cells causes the silver (Ag) salts in the emulsion to

become "exposed", in a similar way to light exposure, and the Ag grains produced will be seen on the emulsion as dark spots. The presence of these dark spots simply indicates the ^{14}C presence.

Any radioisotope other than ^{14}C (^{32}P, ^{15}N, ^{3}H (tritium), *etc.*) can, however, be used in a similar process for uptake detection. Different already-labelled compounds, with specific radioisotopes of many different kinds, can be purchased. If the cell suspension or soil sample, with an added glucose, is incubated longer, the glucose will be metabolised and can, thus, be detected after absorption in an apparatus similar to that described for respiration measurements, above. An organic liquid is used in absorbing the CO_2 so that an organic scintillation liquid can directly be added to the absorbing fluid. This can then be placed in scintillation counter and the light flashes emitted by the scintillation fluid molecules, immediately they are impacted by emitted particles from the radioisotope decay, can be measured. In this way, the radioactivity quantity can also be recorded. Stable (*i.e.* non-radioactive) isotopes can be used in a similar way to trace microbial activity. An example is the use of ^{15}N to later trace N_2-fixation. Another is an incorporation of ^{3}H-labelled thymidine ($C_{10}H_{14}N_2O_5$) into DNA by cells which is a popular measure of growth rates and activity.

3.2.4 Biomass measurement

A measure of the total microbial biomass in soils is often required when studying productivity or fertility of soils. Sometimes the biomass of specific parts of the microbiota is required, for example, fungal versus bacterial biomasses. Earlier biomass determination methods relied on directly counting microbial propagules and other cells either by microscopy or viable plate counting and converting same into biomass through a conversion factor. More recently, however, newer techniques have been developed to use measures of such cell components as adenosine triphosphate (ATP) to estimate the biomass. These techniques include:

a) Soil fumigation method

Fumigation, a process of application of smoke, vapour or for disinfection can be employed to detect and/or measure microbial activities taking place in soils. A soil sample can, for example, be sieved and placed into a container and CO_2 output measured for 20 days. There is typically a period of rapid respiration followed by a lower though stable rate of respiration. If organisms are killed through chloroform fumigation, the initial flush of activities does not take place and the cells are hence killed. If however, the chloroform is removed, there will be even a bigger flush or rapid respiration as the dead microbial cells will contribute to the total available substrates. The difference between the normal rapid respiration and the greater quantity after chloroform treatment is due to the quantity of microbial biomass originally in that system.

b) Determination of adenosine triphosphate content of soils

As a nucleotde used as a source of energy in cellular reactions and, especially, in the synthesis of nucleic acids (DNA and RNA), the presence of adenosine triphosphate (ATP) can be used a basis of detection and/or determination of soil microbes. The ATP is extracted from the cells in the soil and measured by its reaction with the enzyme system luciferin + luciferinase. The enzyme luciferinase is extracted from the tails of firefly which emits light when ATP reacts with its luciferin substrate. The emitted light is now measured in a scintillation counter. Specific machinery available for the ATP determination integrates the reaction/enzyme/substrate system into one

vial of a dedicated small scintillation counter. The amount of ATP per gram of a cell material varies, but averages to 10.0 moles g^{-1} of resting biomass. It has been proposed that the ratio of ATP to biomass C content is 1:120 in soil samples, and this is closer to the ratio in an exponentially growing microorganisms and eucaryotic cells.

c) Determination of cell wall components of bacteria

Bacteria contain such specific cell wall components as muramic acid that can be released by an acid hydrolysis and analysed by a High Pressure Liquid Chromatography (HPLC). Levels of the muramic acid in bacterial cell walls vary, depending on whether the cells are Gram negative or Gram positive (*i.e.* a mean of 12 g mg^{-1} average in Gram negative and 44 g mg^{-1} in Gram positive cells). Thus, unless the Gram positive to Gram negative cells proportion is known, this technique is seriously limited. Bacterial spores are also composed of up to 4 times the normal amounts of muramic acid.

d) Dilution plate counts and direct microscopic counting

Dilution plates are usually only capable of culturing between 1 and 10 % viable soil samples organism. Such direct microscopic observation methods as FITC, acridine orange staining and others usually overestimate the number of cells as they include dead organisms and/or other particles in their counts.

4.0 CONCLUSION

There is no single, all-encompassing, detection and/or isolation technique for soil microbes. A combination of methods may, however, be used with greater benefit. Every additional technique employed, however, further limits the extent of organisms to possibly be obtained. Therefore, obtained experimental results and the interpretations made therefrom depend largely on the isolation techniques employed in deriving the results. It is of great significance, therefore, for the investigator to be conversant with the constraints of each of the techniques used.

5.0 SUMMARY

In this unit, we have learnt that:

1. There are different methods of detection and/or isolation of soil microbes.
2. There are methods for the isolation of specific groups and overall biota in soil.
3. Microbial activities in the soil can generally be measured.

6.0 TUTOR-MARKED ASSIGNMENT

1. Explain the various methods employed in the detection and isolation of soil microorganisms.
2. Which of those methods is unanimously agreed to be the best?

7.0 REFERENCES/FURTHER READING

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UNIT 3 FACTORS AFFECTING ABUNDANCE OF BACTERIA IN SOIL

CONTENTS

1.0 INTRODUCTION

As highlighted in unit 1, all cell forms in life can either be linked to one of the three primary domains of bacteria, archaea and eucarya (Woese and Fox 1977; Woese 1987; Woese *et al*., 1990). According to the rRNA sequences and cultivation methods, twelve bacterial phyla were well defined and delineated (first) by Woese (1987). Soil bacteria, as individuals and communities, have been intensively studied for over a hundred years. There are still, however, more than 85 – 99 % of bacteria that cannot be isolated and cultured (Lok, 2015)

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- recognise the soil bacteria
- know the factors that affect abundance of bacteria in soil
- be introduced to metabolic diversity of bacteria

3.0 MAIN CONTENT

3.1 Soil bacteria

Bacteria, single celled and among smallest living organisms available, outpace all other soil organisms in types and population. The most common soil bacteria are rod-shaped, a micron (*i.e.* 1 /25,000 of an inch) or less in diameter, and up to a few microns long. Researchers' estimate of the live weight of soil bacteria stands at exceeding 2,000 kg ha^{-1}. Most soil bacteria are heterotrophs requiring determined C and obligate aerobes necessarily requiring soil oxygen (O_2) supply to survive. Some are obligate anaerobes hence can only thrive in an absence of O_2. Some bacteria, however, are facultative aerobes thriving well in aerobic environments while they can still adapt to anaerobic environments.

3.1.1 Method of obtaining microbial abundance in soil

Classical plate-counting method, through counting different colonies on the plate, was the easiest method of simultaneously obtaining information on the absolute and relative soil bacterial abundances. The method is, however, limited by the low percentage of bacteria cultivable in soil. Many other techniques have, therefore, been applied in exploring the absolute or relative bacterial community abundances in the soil.

3.2 Factors affecting abundance of bacteria in soil

Generally, the main environmental variables that affect life in soils include pH, aeration, moisture, temperature and organic matter; and such inorganic nutrients as N and P. The abundance of soil bacterial community is, therefore vulnerable to a constant influence of its inherent environment. Changing in any of the factors that affect bacterial abundance will induce a natural selection pressure which, with time, is likely to change its community.

3.2.1 Environmental factors

There are a number of environmental factors that affect the bacterial community. Some of these factors are called modulators and others resources. Examples of the former are temperature, pH, water potential and salinity and for the latter, which are the microbial community needs for growth, include C and N. The organisms compete, actively, for resources but cannot compete for modulators. Microbes can be homeostatic in responding to changes in modulators. If the external pH changes, for example, the internal pH will be maintained. Also, microorganisms will change their membrane fatty acids composition after a temperature change, or may alter their internal solute potential while responding to changes in soil salinity. An altered pressure, due to selection, may eventually cause a change in the bacterial population composition. Depending on difference in temperature regimes, bacteria have different temperature (minimum, optimum, maximum) relationships based on their environmental conditions.

3.2.1.1 Temperature

Soil temperature greatly dictates the levels of biological, chemical and physical processes in the soil. This can normally be is understood the Q_{10} relationship, that is, the factor by which an activity leapfrogs when temperature increased by 10 °C. Within a limited temperatures range the levels of biological and chemical processes usually leap two to three times for every 10 °C temperature increase. Temperature is therefore assumed to affect such other processes as the adaptation rate of bacterial community after a soil disturbance event.

Although most rhizobia have an optimum growth temperature range of 28 to 31 °C in cultures, many are not capable of growing at 37 °C. However, 90 % of cowpea *Rhizobium* strains isolated from hot and dry Sahel savannah environment thrived well at 40 °C. Other soil bacteria also have an optimum growth temperature range of 25 to 30 °C. The apparent, calculated minimum and maximum temperatures, was respectively, around -10 °C and approximately 45 °C. Soil bacteria were better adapted to high temperatures (> 30 °C) than fungi, and vice versa at lower temperatures (< 10 °C).

3.2.1.2 Soil reaction (pH)

Soil reaction, as one of the most crucial environmental factors, also has a dictating function in determining the type of microbes that preponderate soils. Although it does not usually differ much over time, pH can swiftly be changed due to such management practices as liming, in an attempt to counterpoise acidity problems. Liming has, therefore, severally been reported as increasing bacterial population in plate counts. This was due to susceptibility of available C, in acid soils, to microbial attack under liming-raised pH. Liming, therefore, affects the composition of bacterial population and results in a community of alkaline adapted population. . The adaptation of soil bacteria to higher pH, after liming, was mainly temperature dependent, with the fastest change rate at the highest temperature (30 °C). Rate of change in tolerance to pH by soil bacteria was reported as not correlating with the effect of activity due to temperature. This indicates that the adaptation and turnover rates of the bacterial population were not directly correlated.

Low pH is of an especial importance in tropical soils, which are largely acidic and the problem can either arise from problem of survival in a medium of low pH or that of

chemical changes in soil, which is caused by high acidity especially due to large quantities of Al, or Fe and Mn in solution, on one hand, and decreased P and molybdenum (Mo) and the lack of Ca in majority of acidic soils (Giller and Wilson, 1991). Bacterial symbionts are usually affected by low pH although those capable of regulating their internal pH were reported by O'Hara *et al* (1989) to be having an increased survival rate at low pH, for example, some Strains of *Bradyrhizobium* were tested to be more tolerant of Al than *Bradyrhizobium japonicum* strains (Johnson and Wood, 1990).

3.2.1.3 Soil moisture deficiency

Soil water (moisture) is found in soil pore spaces in the form of film. The quantity soil water increases with an increase in soil porosity of soil. Pore spore size heavily depends on the composition of sand, silt and clays otherwise called soil texture. More so, soil moisture is affected through irrigation, drainage or such other management practices as tillage or crop rotation, that enhance the water intake and transmission into the soil. The presence of rhizobial populations in desert soils, vis-à-vis the effective nodulation of legumes growing therein observed emphasise the fact that rhizobia can actual survive in soils limited in moisture levels. Population densities, however, tend to be lowered by the most desiccating conditions and increased with moisture stress. Generally, survival and activity of microbes may rely on changes in soil moisture and their distribution among microhabitats. For example, the distribution of *R. leguminosarum*, in loamy sand and silt loam soils was controlled by the soils initial moisture content. Intermediate moisture tension retarded the movement of *R. trifolii* and migration of bacteria stopped after water-filled pores in soil was halted by water stress.

3.2.1.4 Salinity

Many bacterial species adapt to saline conditions by the intracellular accumulation of osmolytes (*i.e.* organic solutes of low molecular weight). Osmolytes' accumulation is thought to countervail the dehydration effect of low water activity in the medium and not interfering with macromolecular function or structure. Rhizobia utilise this osmotic adaptation mechanism. In the presence of high salt levels, of up to 300 to 400 mM NaCl, the intracellular free glutamate and/or K1 (which strictly controls Mg21 flux during osmotic shock) levels were highly increased, to at times up to six-fold in a few minutes, in cells of, for example, *R. meliloti*, Sinorhizobium fredii and *rhizobia* from the leguminous tree - *Leucaena leucocephala*.

3.2.1.5 Nutrient deficiencies

Deficiencies in essential nutrients needed for the growth of bacteria can cause significant reductions, for example, in the numbers and size of root nodules formed and consequently in the rhizobial population. In acidic soils, as they are usually highly weathered and leached, many such essential nutrients as P and Mo are deficient, because they are bound into forms not available for uptake, whereas such other nutrients as Fe and Zn are unavailable at a high soil pH condition. Deficiencies can however occur in soils of near-neutral pH due to leaching or continuous cropping, and several other nutrients such as micronutrients (like Co, B, Cu or Mo) can be deficient thereby limiting rhizobial population.

3.2.1.6 Pollution

Edwards (1989) and Roberts (1991) reported that at least some of the myriad pesticides used in agriculture can have deleterious effects on the survival of some bacteria. Polluting agricultural soils with sewage sludges that are contaminated with heavy metals has also been shown to drastically and completely suppress N_2-fixation in white clover (*Trifolium repens*) due to the toxic features of the heavy metals to such bacteria as Rhizobium.

3.2.1.7 Biological Constraints

Competition and/or antagonism from other organisms highly influence the growth, survival and abundance of bacteria in the soil.

3.3 Metabolic diversity of bacteria

Bacteria are highly metabolically diverse and can, therefore, be divided into four groups, on the basis of their carbon and energy sources:

3.3.1 *Photoautotrophs*

Like cyanobacteria, this group of bacteria photosynthesise, thereby obtaining their energy from sunlight and carbon by fixing carbon dioxide (CO_2). An example of cyanobacteria in soil is *Nostoc*, which is also a nitrogen (N_2) fixer.

3.3.2 *Photoheterotrophs*

Members of this group derive their energy from photosynthesis if provided with an electron donor (*i.e.* hydrogen or an organic compound) for reductive assimilation of CO_2. Such *photoheterotrophs* as *Rhodopseudomonas*, will grow and thrive on organic substrates if oxygen is made available.

3.3.3 *Chemoautotrophs*

The chemoautotroph members use reduced inorganic substrates to fix CO_2 and as a source of energy. The major energy sources for the organisms in this group are hydrogen (H_2), ammonia (NH_3), nitrite (NO_2), hydrogen sulphide (H_2S) and the ferrous iron (Fe^{2+}). In soil, this group includes the nitrification bacteria (*i.e. Nitrosomonas* and *Nitrobacter*, and *Thiobacillus*, which plays a role in formation of acid mine drainage.

3.3.4 *Chemoheterotrophs*

Organisms in this group require pre-formed organic molecules as their carbon and energy sources. Some bacteria use such simple carbon (C) sources as glucose or succinate, whereas others degrade such more complex substrates as proteins and carbohydrates. Although some bacteria, like *Pseudomonas*, may utilise up to 100 different C sources for growth, majority grow on fewer. Table 2 (below) shows the dominant bacterial phyla in soil environment.

4.0 CONCLUSION

Abundance of soil bacteria is determined by some factors; including biological and environmental.

5.0 SUMMARY

In this unit, we have learnt that:

1. There are various types of bacterial species/strains in the soil, depending on various biotic and abiotic factors.
2. The factors dictating soil bacterial abundance are diverse and sometimes interrelated.
3. Soil bacteria are classified based on their metabolic diversity.

6.0 TUTOR-MARKED ASSIGNMENT

1. Differentiate soil bacteria from other soil microbes in terms of population.

2. Enumerate the various factors dictating the abundance of soil bacteria and explain at least four of them.
3. Write full notes on the metabolic diversity of soil bacteria.

Table 2: Dominant bacterial phyla in soil

Phyla/Subphyla	Mean contribution (%)	Range (%)	Examples
α-Proteobacteria	19	2–43	*Sphingomonas, Rhizobium, Mesorhizobium, Bradyrhizobium, Methylobacter, Methylophilus, Nitrospira,*
β-Proteobacteria	10	2–31	*Burkholderia, Alcaligenes, Acidovorax, Collimonas, Nitrosospira, Thiobacillus, Rhodocyclus, Methylomonas*
γ-Proteobacteria	8	1–34	*Pseudomonas, Xanthamonas, Azotobacter, Thiocapsa, Chromatium*
δ-Proteobacteria	2	0–10	*Desulfovibrio, Bdellovibrio*
ε-Proteobacteria	<1	0–1	*Helicobacter, Campylobacter*
Acidobacteria	20	0–35	*Acidobacterium*
Actinobacteria	13	0–25	*Arthrobacter, Rhodococcus, Streptomyces, Mycobacterium, Rubrobacter, Terrabacter, Acidimicrobium*
Verrucomicrobia	7	0–21	*Chthoniobacter, Opitutus*
Bacteroidetes	5	0–16	*Chitinophaga*
Firmicutes	2	0–7	*Clostridium, Bacillus, Lactobacillus*
Chloroflexi	3	0–16	
Planctomycetes	2	0–8	
Gemmatimonadet	2	0–4	*Gemmatimonas*
Other groups	5	2–10	
Unknown	2	0–13	

Source: Janssen (2006)

7.0 REFERENCES/FURTHER READING

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UNIT 4 THE RHIZOSPHERE MICROBES

CONTENTS

1.0 INTRODUCTION

Plant rhizosphere, the soil nearest to the plant root system, is where the roots release large amount of metabolites from the living root-hairs and/or fibrous root systems. The metabolites act as chemical signals for available motile bacteria to move to the root surface. It also represents the main nutrient source available for supporting the growth and persistence. Some of the microorganisms that inhabit rhizosphere are bacteria that are capable of colonising the roots or the rhizosphere soil of crop plants very efficiently. These bacteria are called plant growth promoting rhizobacteria (PGPR). They fulfil important functions for plant health and growth. Various mechanisms are at play in suppression of plant pathogens, usually indirectly connected with plant growth.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain what rhizosphere is and it differs from edaphosphere
- describe the types of rhizosphere organisms available in the soil.

3.0 MAIN CONTENT

3.1 Rhizosphere

The term ‘‘rhizosphere’’ was derived from the Greek word ‘rhiza’, which means root, and ‘sphere’, meaning a field of influence. It has first been defined by a German scientist Hiltner in 1904 as ‘‘the zone of soil immediately adjacent to legume roots that supports high levels of bacterial activity’’. Over time, however, it has now been severally redefined to include the soil volume that is influenced by the root and parts of its tissues vis-à-vis the soil that surrounds the root where physical, chemical and biological properties are changed by the growth and activity of the root (Pinton *et al*., 2001). Rhizosphere has broadly been subdivided into three zones as indicated in Figure 7 below:

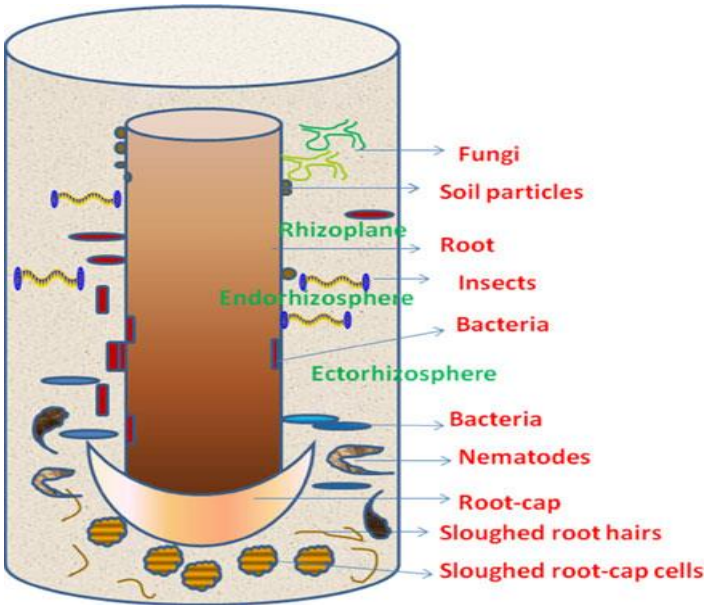

Figure 7 A simplified diagram of rhizosphere. Source: Prashar et al. (2013).

Endorhizosphere
This area consists of the root tissue that includes endodermis and cortical layers.

Rhizoplane
This is the root surface where microorganisms and soil particles adhere, and it consists of cortex, epidermis and mucilaginous polysaccharide layer.

Ectorhizosphere
This area consists of the soil immediately adjacent to the root.

Apart from the aforementioned basic zones, some other layers may, in certain cases, also be defined. For example, in mycorrhizal association, there is a zone called mycorrhizosphere while in some other plants, there is a strongly adhering dense layer termed rhizosheath. Rhizosheath consists of the root hairs, mucoid material, microorganisms and soil particles The root itself is a part of the rhizosphere as endophytic microbes also colonise the inner root tissues. The soil volume which is not a part of the rhizosphere (*i.e.* which is not at all influenced by the root) is referred to as bulk soil or edaphosphere. Dead roots are transformed into soil by a rhizospheric activity and it is, therefore, different from the bulk soil. Rhizosphere may, thus, be regarded as a unique region that differs from the bulk soil.

3.1.1 Rhizosphere effect

Plants, in due course of their growth and development, pass through the early seed germination and seedling growth stages, during which process a variety of organic compounds are exuded, secreted and deposited by the roots. This makes the rhizosphere rich in nutrients when compared to the bulk soil. This promotes the setup of active and enhanced populations of microbes in the root zone than the bulk soil. This phenomenon of establishing a rich microflora, in the rhizosphere under the influence of root-secreted nutrients, is known as the rhizosphere effect or (alternatively) plant effect. The rhizosphere (or plant) effect is, however, calculated in terms of rhizosphere ratio (R: S) achieved by dividing the total number of microbes in in the rhizosphere (R) by the corresponding number in the bulk soil (S).

Rhizosphere effect is reflected by the observable difference in the microbial populations' structure of uncultivated and cultivated soils and the variations in the structures of bacterial and fungal communities with such rhizosphere related factors as crop variety, plant growth developmental stages and soil characteristics. Although a higher rhizosphere effect was reported for bacteria, showing R: S values a range between 10 and ≥ 100 than with fungi,

recent studies that utilised cultivation-independent analysis of soil microflora have also indicated significant rhizosphere effect for soil the fungi. For some classes of soil bacteria, such as ammonifying and denitrifying bacteria, an even more pronounced rhizosphere effect has been noticed but almost negligible for algae.

3.1.2 Rhizodeposition

The term rhizodeposition, as first defined by Whipps and Lynch (1985), is the material that is lost from plant roots, including water-soluble exudates, insoluble secreted materials, lysates, dead fine roots and gases such as CO_2 and ethylene (C_2H_4). It can simply be defined as organic compounds that are released, by the roots of living plant, into their surrounding environment which may also include inorganic ions. It is equivalent to nearly 15 – 60 % of the total photosynthates of the plant which leads to an accumulation of substantial reserves of C and energy in the rhizosphere for the microflora. The plant-derived C allocated belowground through roots, consists of three major components, as follows:
1. Roots mass: that may be living or dead; 2. Rhizodeposit: materials of plant origin localised in the rhizosphere or the surrounding soil utilised and transformed by rhizosphere biota and mixed with soil organic materials; and 3. Carbon dioxide: released as a result of respiration of roots and root symbionts or microbial respiration.

In rhizosphere C fluxes studies, rhizodeposition is a very significant process. Rhizodeposit is also subdivided into various parts (Figure 8), including: root cap cells and root tissues, mucilage and root exudates. Root exudates are the most important part of the rhizodeposit and are classified into two kinds, depending on their molecular weight (low and high).

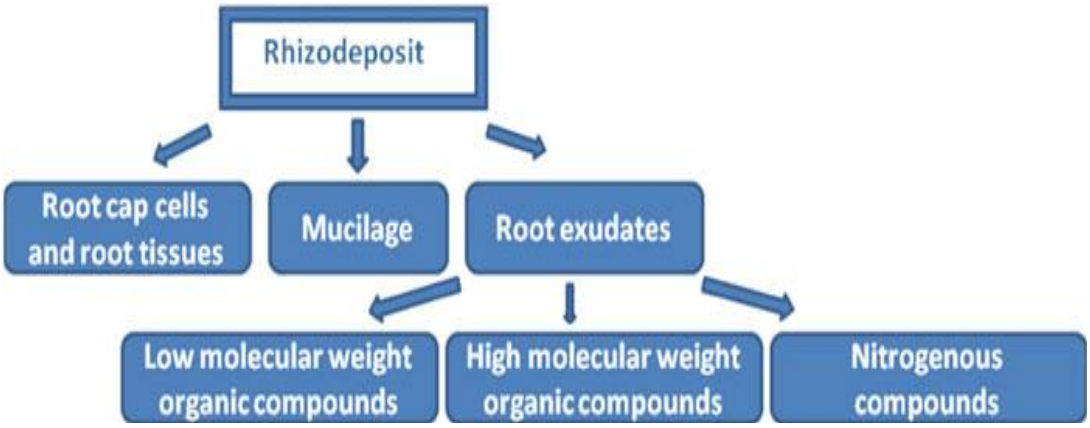

Figure 8 Rhizodeposit consisting of plant-origin material released by roots and localised in the rhizosphere. Source: Prashar et al. (2013).

Examples of class one (low molecular weight components) include such H_2O-soluble compounds as amino acids, organic acids, simple carbohydrates, plant hormones, vitamins, sugar phosphate esters, phenolics, ions and many other C-containing secondary metabolites. The high molecular weight (the second class) exudates generally include mucilage (polysaccharides), enzymes and proteins. The latter (high molecular weight exudates) proved more significant based on the total mass of the root exudates, although have comparatively lesser variety than the class one members.

The exudates may, however, also be classified into active and passive, based on the role they play and the mode of their secretion by roots. Passive exudates possess unknown functions and are diffused as basal exudation from the roots (output of waste materials) depending on gradient. They form around 3 – 5 % of the total C fixed during photosynthesis. The active exudates, on another hand, are secreted via plants' open membrane pores and have such a specific function as lubrication and defence. Exudates can also be classified on the basis of their biological activity (like being phytohormones, enzymes, signalling molecules, phytoalexins or allelochemicals), and the chemical composition of their rhizodeposition. Exudation provides a wide array of physical and chemical advantages to the plant,

including: friction reduction between root tips and soil, reduction in root desiccation process and improvement of soil structural stability.

3.2 Rhizosphere microbes

Dommergues classified rhizosphere microorganisms as either beneficial (symbiotic), harmful (pathogenic) or having no effect on the plant (neutral). Pathogens can either be major (or true) pathogens that penetrate stele and destroy the phloem, thereby causing major disease symptoms, or minor pathogens, either parasites or saprophytes, which are confined to such juvenile plant tissues as cortical cells, root hairs and root tips. Minor pathogens include such obligate and facultative parasites as *Polymyxa* and *Olpidium.* The minor pathogenic rhizosphere microorganisms whose metabolites affect plants without parasitizing plant tissues are referred to as deleterious rhizosphere microorganisms (DRMO). The DRMO should, however, be distinguished from those that are parasitizing plant tissues, called parasitizing minor pathogens (PMP). The DRMO include deleterious rhizobacteria (DRB) and deleterious rhizofungi (DRF). The pathogenicity of DRMO is difficult to demonstrate as effect on plants is mostly restricted to retardation in root and/or shoot growth without any other distinct symptoms.

3.2.1 Diversity of rhizosphere microbes

The rhizosphere microflora, therefore, includes bacteria, fungi, protozoa, algae, nematodes and microarthrops. Up to 98 % of the microbes in soil cannot be cultured. Therefore, their identification, characterization and description of their role are particularly herculean. Nucleic acid based techniques, including analysis of DNA and rRNA molecules from soil samples have recently revealed enormous diversity in the microbial flora inhabiting the rhizosphere. The number of soil microbial species may vary from thousands to millions. Many studies suggest that Proteobacteria and the Actinobacteria form the most common and dominant populations, of up to ≥ 1 % in the rhizosphere of many plant species. These groups are the most studied rhizobacteria, and as such, contain the majority of organisms investigated, both as inoculants and pathogens.

3.2.1.1 Influence of root exudates

Specific root exudates content of may create a niche that influences the microorganisms that colonize the rhizosphere, thereby changing the diversity and composition of microorganisms that colonise the rhizosphere in a plant specific way. Plant species, plant developmental stage and soil type have thus been indicated as major factors determining the composition of rhizosphere microbial communities. Plant and/or soil type are also reported, by some authors, as the dominant factor(s). Based on this therefore, it can generally be deduced that diversity and predominance of microbial population in the rhizosphere depend on some biotic and abiotic factors that are prevailing in a given ecological niche.

3.2.2 Population level

Growth media studies steadily indicated that rhizosphere bacterial populations are several orders of magnitude larger than those in the bulk soils (edaphosphere). Rhizosphere bacteria concentration can reach up to between 10^{10} and 10^{12} cells per gram of soil, and are transferred to various related environments, including animals, plants, foods, freshwater and marine habitats. Only a few groups of these bacteria are, however, considered to be soil borne due, probably, to the fact that non-spore forming bacteria cannot thrive in soil for long periods. Microorganisms inhabiting the rhizosphere compete with each other for water, space and nutrients. They sometimes improve their competitive efficiency by developing a closer association with plant. This process can be regarded as an on-going process of micro-evolution in low-nutrient environments, which are very common in natural ecosystems.

3.2.3 Beneficial microorganisms and modes of action

Plant-beneficial microbial interactions can be roughly divided into three categories:

- Those microorganisms that are, in association with plants, responsible for its nutrition (*i.e.*, microorganisms that can increase the supply of mineral nutrients to the plant). In this case, while most may not directly interact with the plant, their effects on soil biotic and abiotic parameters certainly have an impact on plant growth.
- There is a group of microorganisms that stimulate plant growth indirectly by preventing the growth or activity of pathogens. Such microorganisms are referred to as biocontrol agents, and they have been well documented.
- Third group involves those microorganisms responsible for direct growth promotion, for example, by production of phytohormones.

There has been a lot of literature describing the potential uses of plant-associated bacteria as agents that stimulate plant growth and manage fitness of soil and plant. On another hand, apparently neutral interactions are extensively found in the rhizosphere of all crop plants. Saprophytic microorganisms are responsible for many such crucial soil processes as decomposition of soil organic residues and associated nutrient turnover or mineralisation processes. Whereas these organisms do not seem to directly benefit or harm the plant, hence being neutral, their presence is actually important for soil dynamics, and their absence would vividly influence plant health and consequent productivity.

Rhizobacteria (Rhizosphere-living bacteria) are a subset of the total rhizosphere bacteria which have the capacity, upon re-introduction to seeds or vegetative plant parts, to colonise the developing root system in the presence of competing soil microflora. Those types of rhizosphere-living bacteria which negatively affect the plant are termed deleterious rhizobacteria while those that influence the plants positively are called plant growth promoting rhizobacteria (PGPR). Recently, the number of bacterial species identified as PGPR increased due to numerous studies that covered a wider range of plant species and the advancements in bacterial taxonomy vis-à-vis the progress made in the understanding of the various mechanisms of PGPR action. The PGPR modes of action in promoting plant growth, development and protection involve complex mechanisms. Important the mechanisms are: biofertilisation: which involves increasing the nutrients availability to plant, phytostimulation: which involves promotion of plant growth, usually by the production of phytohormones and biocontrol: involving diseases control, majorly by production of antifungal metabolites and antibiotics, lytic enzymes and induction of plant defence responses. *Bacillus* and *Pseudomonas* genera are the PGPR that are most commonly investigated, and mostly the dominant bacterial groups in the rhizosphere.

The most important pathogen groups in the soil adversely affecting plant health and growth are fungi and nematodes. Bacterial (deleterious rhizobacteria) and viral pathogens are lesser known in causing root infections as they not capable of infecting the intact root tissue, besides they require an opening to penetrate the plant. More so, nonspore-forming bacteria are not capable of surviving in soil for longer periods. Deleterious rhizobacteria may produce diverse kinds of phytotoxins and also present competition for nutrients and inhibition of mycorrhizal fungi.

3.2.4 Colonisation

In all successful plant-microbe interactions, the competence to colonise plant habitats is important. Single bacterial cells can attach to surfaces and, after cell division and proliferation, form some dense aggregates commonly known as biofilms or macrocolonies. Colonization steps include: attraction, recognition, adherence, invasion (only for endophytes and pathogens), colonisation and growth, and many strategies to establish interactions.

4.0 CONCLUSION

Rhizosphere is the zone of soil that surrounds plant root, where biological and chemical activities in the soil take place, under the root influence. It has a rich pool of potential sources of bacteria that are equipped with versatile abilities of favourably influencing their host plant. Bacteria are, therefore, the most abundant organisms available in rhizosphere and a special bacterial class called plant growth promoting rhizobacteria (PGPR) influence plant growth by a number of mechanisms, direct and indirect, in a wide array of crop plants. As plant roots grow through the soil they secrete such water-soluble compounds as sugars, amino acids and organic acids that supply the microorganisms with nourishments.

5.0 SUMMARY

In this unit, we have learnt that:

1. Rhizosphere is broadly divided into some zones.
2. Rhizosphere organisms are of various diversities and types and playing different roles.

6.0 TUTOR-MARKED ASSIGNMENT

Discuss the rhizosphere microorganisms based on diversity, type and/or function in soil and crop plants.

7.0 REFERENCES/FURTHER READING

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UNIT 5 NITROGEN CYCLE

CONTENTS

1.0 INTRODUCTION

Nitrogen is present in various forms, primarily as dinitrogen gas (N_2), organic and inorganic, as ammonium (NH_4^+) and nitrate (NO_3^-) ions. These forms can, however, be transformed from one form to another by some microbially mediated processes. Nitrogen cycle is a biogeochemical cycle in which nitrogen (N) is converted into many chemical forms while circulating between the atmospheric, terrestrial and aquatic environments. Conversion of N can be accomplished via both physical and biological processes. Important processes involved in N cycling include fixation, ammonification, nitrification and denitrification. Majority (up to 78 %) of the Earth's atmosphere is composed of N thereby making atmosphere the largest N source. The atmospheric nitrogen (N_2), however, has a biologically limited availability for use which leads to the noxious scarcity of the usable N forms in many ecosystems.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- describe the various stages and functions of nitrogen cycle
- state how nitrogen leaves the soil
- enumerate the components of nitrogen cycle. Explain any three of them
- describe the sources of nitrogen to soil

3.0 MAIN CONTENT

3.1 Nitrogen

Nitrogen (N) is one of the nutrient elements that are universally limiting in agricultural lands due to its unavailability, even though among the most abundant elements on the earth. Only sunlight and water are more important. Plants acquire N from two principal sources: (a) the soil, through commercial fertilisers, manure and/or mineralisation of organic matter; and (b) the atmosphere, through symbiotic N_2 fixation. Plants can accumulate N in their vegetation for periods of up to hundreds of years, as in trees, or cycle it seasonally in annual crops.

3.1.1 Forms of nitrogen

Nitrogen pools vary in size over several orders of magnitude. The relatively inert nitrogen pool tends to be ignored, possibly because it is an invisible gas, yet it is actually the largest pool of biologically active N in terrestrial eco-system. Soil organic N is the next largest N pool and varies widely among soil types. The variation is largely determined by soil

forming factors, especially temperature and precipitation. The amount of N tied up in plant biomass is of intermediate size and varies as a function of vegetation type (*example.* forests, versus grassland), climate and soil N availability. Soil inorganic N pools are usually small, generally just a few mg N kg^{-1} in natural ecosystem and rarely exceeding 100 mg N kg^{-1} in the plough layer of recently fertilised agricultural soils. Larger N pools tend to be less reactive (that is, they turn over more slowly) whereas smaller pools are usually more dynamic. For example, N in plant biomass often turns over annually, while inorganic N pools are so dynamic that they may turn over more than once in a day.

i. Soil organic nitrogen (SON)

The N contained in soil organic matter (SOM) occurs in a wide range of compounds, of which only about half can be identified definitely. Naturally occurring organic-N compounds isolated from soils include proteins and amino acids (*e.g. Alanine, Phenylalanine, Aspartic acid, Arginine, Glycine, Glutamic acid, Lysine, Tyrosine and Tryptophan*), microbial cell wall polymers and amino sugars (*Glucosamine*), nucleic acids (*Uracil, Cytosine, Thymine, Adenine and Guanine*) and various vitamins, antibodies and metabolic intermediates. As much of the organic N in soil is of unknown composition, a fractionation procedure based on acid hydrolysis has been used to characterise SON. The range of amino sugar N mainly found in microbial cell walls is similar to that usually found in microbial biomass N, which is usually about 5% of total soil N.

Organic N that is, for example, found in manures is generally assumed to have not possibly been utilized by plants until it is converted into an inorganic form. It was also thought that microorganisms use organic N compounds just a source of carbon but not N. However, recent studies revealed that organic N compounds may directly be used as a source of N for soil microorganisms as well as for plants, particularly those plants that have their roots colonized by certain types of mycorrhizal fungi.

ii. Soil inorganic nitrogen

Unlike SON, the important inorganic N forms in soil ecosystem are well characterised, primarily because most inorganic N compounds can be readily separated and measured. Inorganic N pools in soil are nevertheless important as they serve as substrates, metabolic intermediates, alternate electron acceptors or products of the many biological N transformations. Some important inorganic N forms are as shown on Table 3 below:

3.1.2 Transformations of nitrogen

More time and efforts have, perhaps been invested in N-cycle studies than any other soil microbiological topic of study. This may be due to the importance of N as an essential nutrient for earthly life. It is the most limiting nutrient to plant growth in terrestrial ecosystems, and so N-cycle affects the environment in various ways. For example, there are currently serious concerns about high nitrate (NO_3) concentrations in ground and surface waters and the contribution of such gaseous nitrogen oxides as No and N_2O, to large–scale environmental problems of acid rain, ozone depletion and greenhouse warming. Large diversity of N-containing compounds, which exist in numerous oxidation states, and the wide array of microbial transformations make N-cycle an extremely interesting intellectual challenge.

3.1.3 The nitrogen cycle

Nitrogen is present in various forms-primarily as dinitrogen gas (N_2), organic, and ammonium (NH_4^+) and nitrate (NO_3^-) ions. These can be transformed from one form to another by some microbially mediated processes. Components of the N cycle include ammonification/mineralisation, immobilisation, N_2-fixation, nitrification and denitrification. An overview of the N-cycle is as presented in Figure 9 below:

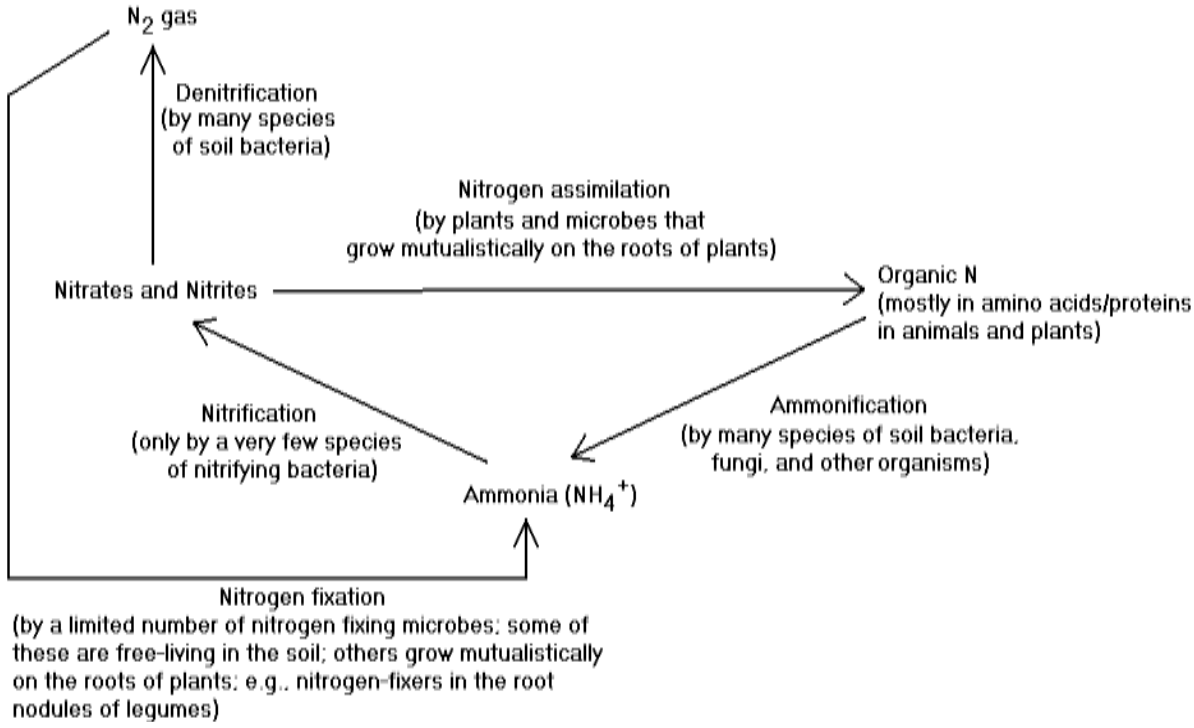


Figure 9. Overview of the Nitrogen cycle showing major pools cycled and transformations (lines) of N

1. Nitrogen mineralisation

As microorganisms decompose organic matter, ammonium (NH_4^+) is released in a process called mineralisation. The amount of N converted from organic forms to available (inorganic) forms through mineralisation ranges from 15 to 69 kg ha^{-1} $year^{-1}$. Mineralisation amounts are higher in soils with higher amount of organic matter. As a rule of thumb, however, 22 - 34 kg N ha^{-1} is mineralised per 1 % organic matter. However, many studies have shown that only about 1.5 - 3.5 % of organic N is annually mineralised. This is because mineralisation requires microorganisms and it is, as such, highly affected by such soil conditions as aeration, moisture, temperature, pH, *et cetera*. The small N produced through mineralisation still provides sufficient mineral N for normal growth of natural vegetation in most soils except in sandy soils due to their low organic matter content. Rough estimate of the quantity of N to possibly be mineralised can be made, provided the OM content of a soil is known. Mineralisation can, however, be broadly seen in general terms as the production of inorganic N (both NH_4 and NO_3) or more narrowly, as the production of NH_4. The increase (or sometimes decrease) in inorganic N is most often called" net N mineralisation" as it represents the sum of simultaneous ammonium production and consumption processes. It is; therefore, more correct to use the term *ammonification* or *gross N mineralisation* to mean the biological transformation of organic N to NH_4.

2. Immobilisation

Immobilisation almost always means the conversion of NH_4 to organic N, mostly due to the assimilation of NH_4 by the microbial biomass. Immobilisation, therefore, temporarily renders the N unavailable for plants and/or microbes. Immobilisation may sometimes refer to the assimilation of both the NH_4 and NO_3. Assimilation of the latter is usually termed NO_3-immobilisation and it requires reduction into NH_4 before the N can be incorporated into cell constituents. The enzymatic process of mineralisation/immobilisation can be illustrated as indicated in Figure 10.

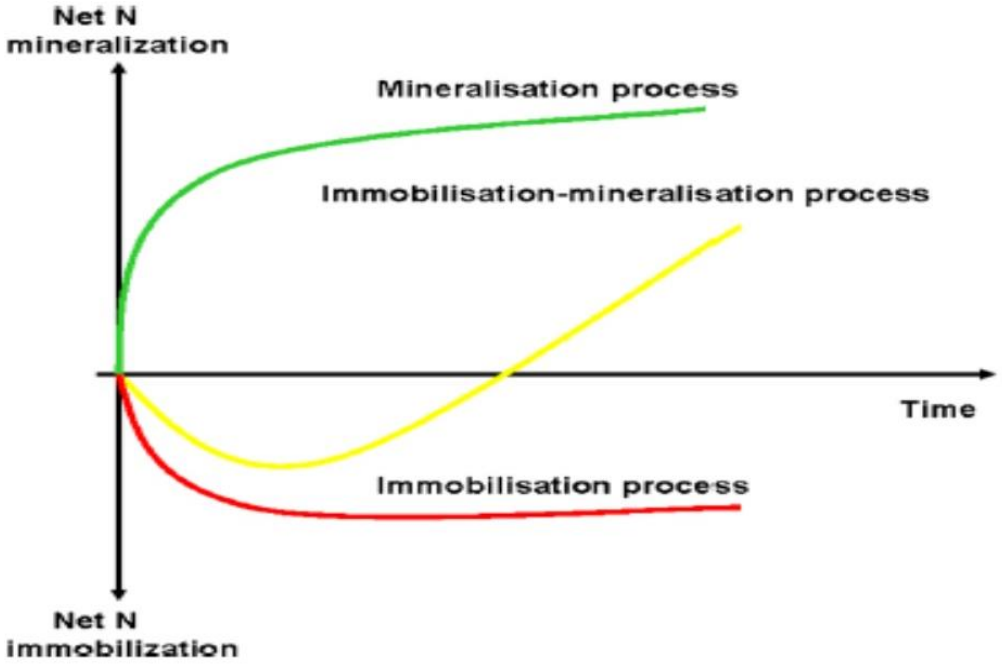


Figure 10. An illustration of mineralisation/immobilisation process. Source: Chen et al. (2014).

NB: for mineralisation to occur, C: N ratio must be ≤ 20

3. Immobilisation/Assimilation

Microbes and other organisms assimilate NH_4 by two major pathways: glutamate dehydrogenase and glutamine synthetase-glutamate synthase (GOGAT). When NH_4 is present in relatively high concentration (> 0.1 mm or about 0.5 mg N kg^{-1} soil), glutamate dehydrogenase, acting with NADPH2 as a coenzyme, can add NH_4 to α-ketoglutarate to form glutamate, as shown in Figure 11. Ammonium is present at low concentrations in most soils, this result in low intracellular NH_4 concentration. The second NH_4 assimilation system comes into operation under these conditions. The GOGAT pathway is complex. The first step requires ATP to add NH_4 to glutamate to form glutamine, while the second step transfers the glutamine ammonium to α-ketoglutarate to form two glutamates. When incorporated into glutamate, the NH_4 can then be transferred to other carbon skeletons by various trans-aminase reactions in order to form additional amino acids. It is important to realise the fact that net mineralisation of NH_4 takes place in N-limiting conditions, otherwise net production occurs.

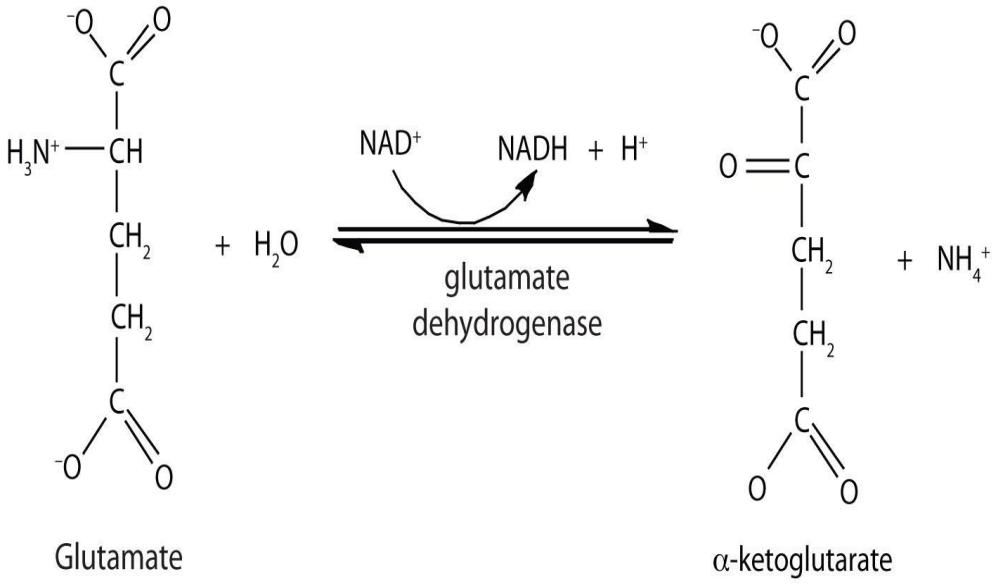


Figure 11. Showing glutamate formation.

4. Ammonification

This is the conversion of organic-N compounds to NH_4, and mediated by enzymes produced by microbes and other soil animals. Ammonification involves several steps, in that, extracellular enzymes first breakdown organic-N polymers such that the resulting monomers pass through across the cell membrane and further metabolised, with the consequent production of NH_4 which is released into the soil solution. Microbial cell walls are thought to relatively be recalcitrant in soils, yet several common extracellular enzymes are capable of degrading them. Chitin, for example, which forms the cell wall of many fungi and part of exoskeleton of insects, is degraded by the combined activities of chitinase and chitobiase. Chitinase breaks chitin, a polymer of N-acetylglucosamine, into dimers (chitobiose), which are subsequently cleaved to two molecules of N-acetylglucosamine by chitobiase. Nucleic acids are degraded by ribonucleases (RNases) and deoxyribonucleases (DNases), urease, another important extracellular enzyme, hydrolyses urea into CO_2 and NH_3. In most cases, the final NH_4 production occurs within microbial cells through the action of intracellular enzymes. Some of these intracellular enzymes may become "extra cellular" when microbial cells are lysed.

Table 3: Forms of inorganic nitrogen in the soil

Compound	Formula	Oxidation State	Form in Soil	Major Attributes
Ammonium	NH_4^+	-3	Fixed in clay lattice, dissolved, as gaseous NH_3	Cationic, rather immobile, volatile at high pH, assimilated by plants and microbes.
Hydroxylamine	NH_2OH	-1	Not detected	Intermediate in NH_3 oxidation.
Dinitrogen	N_2	0	Gas	Largest pool of N, relatively insoluble, substrate of N_2-fixation, end product of denitrification.
Nitrous Oxide	N_2O	+1	Gas, Dissolved	Greenhouse gas, very soluble, immediate in denitrification, by-product of nitrification.
Nitric Oxide	NO	+2	Gas	Chemically reactive, intermediate of denitrification, by-product of nitrification.
Nitrite	NO_2	+3	Dissolved	Present at very low concentration, toxic product of NH_3 oxidation, substrate of NO_2 oxidation, intermediate in denitrification.
Nitrate	NO_3	+5	Dissolved	Anionic, mobile, readily leached, assimilated by plants and microbes, end product of nitrification, substrate for denitrification.

Source: Sylvia et al. (2005).

5. Fate of ammonium in soil

Other than the mineralisation/immobilisation cycle, NH_4 has many facts in soil. It can chemically be held on cation exchange sites or become fixed in the lattice of clay minerals (termed NH_4-fixation), such as illite, vermiculite. It may also react chemically with such organic compounds as quinones, or may be volatilised at high pH. Biologically, major fates of NH_4 are plant uptake, microbial assimilation or oxidation to nitrate (NO_3^-) by nitrifying microorganisms.

6. Nitrification

This is the microbial production of nitrate (NO_3^-) from the oxidation of reduced N compounds. In other words, it can be defined as the biological oxidation of NH_4 to nitrites and the further oxidation of the nitrites to nitrates. It therefore involves the biological conversion of N compounds (organic or inorganic) from reduced form to a relatively more oxidised form. The study of nitrification in soil is very important to soil fertility, besides the potential adverse impact that NO_3^- and its denitrification products can have on our environment. Winogradsky established that nitrification is typically associated with certain chemoautotrophic bacteria that are obligate aerobes that derive their carbon solely from CO_2 or carbonates and their energy from the oxidation of NH_4^+ or NO_2^-. The bacteria are classified into two groups, based on whether they oxidise NH_4^+ to NO_2^-, or NO_2^- to NO_3^-, this is the *nitrosomona*s and *nitrobacter* respectively. Table 4 shows the list of chemoautotrophic N oxidisers as thus:

Table 4: Showing the list of chemoautotrophic nitrogen oxidisers

Genus	Species	Habitat
Oxidise NH_3 to NO_2^-		
Nitrosomonas	*europaea*	Soil, H_2O, sewage
Nitrosopira	*briensis*	Soil
NItrosococcus	*nitrosus* *oceanus* *mobilis*	Marine Marine Soil
Nitrosovibrio	*tenuis*	Soil
Oxidise NO_2^- to NO_3^-		
Nitrobacter	*Winogrdskyi* *(agilis)*	Soil Soil, water
Nitrospina	*gracilis*	Marine
Nitrococcus	*mobilis*	Marine

Source: Sylvia et al. (2005).

From "Bergey's Manual of Determinative Bacteriology" 8th (Edition) conversion of NH_3 to NO_3^- by oxidation actions of *nitrosomonas,* the NH_3-oxidising bacteria and *nitrobacter*, NO_2^--oxidising bacteria, constitute the first step of *chemoautotrophic* nitrification. Other microbes can produce NO_2^- and NO_3^- by enzymatic oxidation processes not linked to microbial growth. Many methane-oxidising bacteria genera, for example, contain a membrane bound methane monooxygenase enzyme that will oxidise NH_3 as well as methane (CH_4). The overall reaction for the conversion of NH_3 to NO_2^- is:

$NH_3 + 1.5O_2 \rightarrow NO_2^- + H^+ + H_2O$, however, the oxidation of NO_2^- to NO_3^- is a one-step reaction, that is:

$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

Nitrous oxide (N_2O) and acidity are two other products of NH_3 oxidation. Many heterotrophic microbes also oxidise either NH_4^+ or organic N to NO_2^- or NO_3^- such heterotrophic nitrifiers include fungi (*example, Aspergillus*) and bacteria (*example,*

Arthrobacter spp., some *Actinomycetes, Alcaligenes, etc.*). *Thiospaera pantotropha* and *Paracoccus pantotrophus* can also denitrify in aerobic conditions.

7. Factors affecting nitrification in the environment

Traditionally, soil perfusion apparatus has been used to study the factors that affect nitrification. A metabolite is continuously percolated through a soil column. The repeated perfusion permits direct study of kinetics of the transformation and effects of environmental change. Soil columns receiving water and substrate through multi-channel pumps and interfaced with computer-controlled sampling devices and now also available, such that the soil processes can be measured on a continuous basis.

i. Soil reaction (pH)

There is a strong correlation between NO_3^- production and the soil pH. Optimum pH value may vary between the pH 6.6 to 8.0. In agricultural soils, nitrification rates decrease below 6 and become negligible below 4.5. At high pH, however, NH_4^+ inhibits the NO_2^- transformation to NO_3^- due to the inhibition of action of nitrite oxidisers relative to the NO_3^- oxidisers. *Nitrosospira* and *nitrobacter* are the common genera observed after isolation of several NH_3^+ - and NO_3^- -oxidising bacteria from low pH soils.

ii. Aeration

Since O_2 is an obligate requirement for all species concerned, aeration is essential for nitrification. Diffusion of oxygen (O_2) into the soil, and hence aeration is controlled by such factors as soil moisture and soil structure.

iii. Soil moisture

As soil moisture affects aeration regime of the soil, its water status has an influence on NO_3^- production. Water-logging limits O_2 diffusion, and hence nitrification suppressed. On the other hand, proliferation of bacteria is retarded by an insufficiency of water. Although optimum moisture level varies from soil to soil, in most soils nitrification readily proceeds at -0.1 to -1 Mpa moisture tension mineralisation reactions that produce NH_4^- are less sensitive to both water stress and low temperature: NH_4^- therefore accumulates in water-stressed or cool soils.

iv. Temperature

Nitrification is affected by temperature. The process is slow at below 5 ^{o} C and above 40 ^{o} C. The optimum is around 30 – 35 ^{o} C. The interaction of temperature, moisture, aeration, and other factors makes up the seasonal effect. Nitrification is greatest in spring and falls and slowest in summer and winter in temperate areas.

v. Organic matter

It was, for a long time, thought that organic matter was toxic to nitrifiers but the process occurs in manure piles, thereby debunking the idea. It is therefore now believed that organic matter, per se, is not inhibitory but its decomposition may require inorganic N and O_2, thus depleting supplies of available NH_4^+ and O_2 for the nitrifiers.

vi. Allelochemical inhibitors

Tannins and polyphenols are among the more important allelochemical agents that are thought to cause low nitrate intake concentration in soils of climax communities in natural ecosystems. It was however, realised that active competition for NH_4^+ by plant uptake and mineral mineralisation are probably more dominant cause of that low NO_3^- concentrations than the allelochemicals.

vii. Substrate availability

Substrate availability (particularly NH_4^+) is the most important nitrification regulating factor after the provision of desirable aerobic conditions. The substrate availability is often limiting

to growth and activity of the nitrifiers. As autotrophic nitrifiers mostly dominate nitrification activity, CO_2 concentrations may also influence the growth of nitrifiers. Thus the high CO_2 concentrations found in soils compared to the atmosphere may be beneficial to such nitrifying bacteria, in as far as O_2 does not become limiting. Carbonate (CO_3) equilibrium may also help in poising the soil pH at a more favourable level for the nitrifiers.

viii. Other factors: include fertilisers, type of clays, pesticides, *et cetera*.

8. Fate of NO_3 in the soil environment

Like NH_4^+, NH_3^- has many competing fates in the soil ecosystem, including leaching because it is an anion, NO_3^- can also be assimilated by plants and microorganisms, although plant use NH_4^+ more readily than NO_3^- as the latter has to further be reduced before the plants can actually utilise them. Nitrate can also be reduced by dissimilatory process. In acidic soils, of pH $\leq$ 5, N gases can be produced chemically, with the formation of nitric oxide (NO). Nitrite (NO_2^-) can also react with the amino group of organic nitrogen compounds to form dinitrogen gas (N_2). Nitrate (NO_3^-)-respiring bacteria convert NO_3^- to NO_2^- under anaerobic conditions. In doing so, they gain energy through oxidative phosphorylation (161 KJ or 38 Kcal/mole NO_3^-).

9. Denitrification

Denitrification can be defined as the microbial reduction of nitrate NO_3^-s and NO_2^-s to liberate molecular N and nitrous oxide. The organism that carry out this process are usual present in large number and are more often than not facultative anaerobic bacteria in such genera as *Pseudomonas, Bacillus, Micrococcus* and *Achromobacter*. These are heterotrophs, which obtain their energy and carbon from the oxidation of organic compounds as shown below:

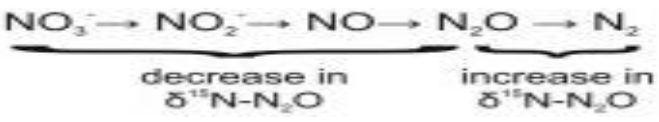
$$24HNO_3 + 5\,C_6H_{12}O_6 \longrightarrow 12N_2 + 3CO_2 + 42H_2O + \text{energy}.$$

In most soils, anaerobic respiration occurs mostly after rainfall as the soil pores become water-saturated and so O_2 diffusion into micro sites drastically slowed. At water-filled pore-space concentrations of 60 % and higher, the overall stoichiometry of the reaction is as thus:

$$2NO_3^- + 5H_2 + 2H+ N_2 + 6H_2O.$$

Organisms denitrify to generate energy (ATP) by electron transport phosphorylation via the cytochrome system.

The general pathway is:



Each step is enacted by individual enzymes, nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos). Each inhibited by oxygen and the organisation of these enzymes in the cell membrane from gram negative bacteria is described in Figure 12. At any step in this process, intermediate product can be exchange with the soil environment, making denitrifiers a significant NO_2^- source in soil solution and an important source of atmospheric gases nitric and nitrous oxides.

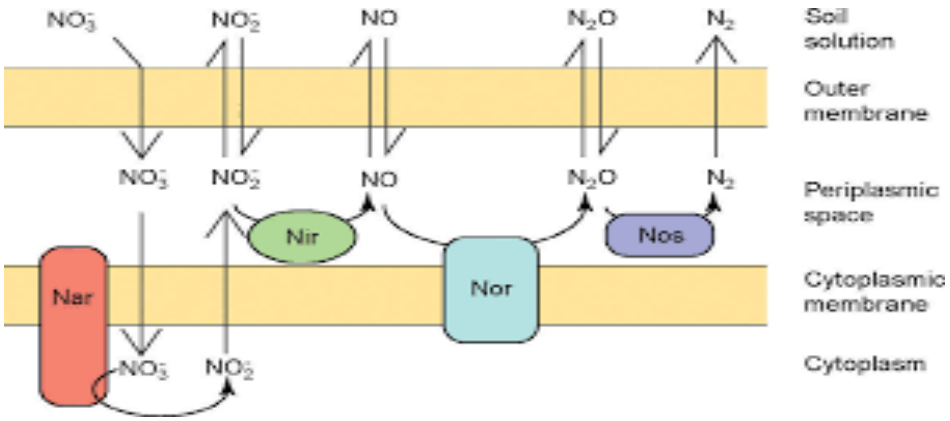


Figure 12. Denitrification pathway showing the location of the four (4) denitrification enzymes: Nar, Nir, Nor and Nos. Source: Robertson and Groffman (2015).

Factors that affect denitrification in the environment include: soil aeration, NO_3^- availability, carbon availability and other miscellaneous soil and environmental factors (*example.* pH and temperature).

4.0 CONCLUSION

Nitrogen exists in various forms, both inorganic and organic, in soils and changes, constantly, from one form to another. The pathways that the different forms of N follow through in the ecosystem are collectively referred to as the nitrogen cycle. Appreciating how these different N pools interact and the various processes by which they enter and leave the cycle is an important subject worthy of continuing study.

5.0 SUMMARY

1.There is more than one form of nitrogen in the soil environment
2. These forms can be changed from one form to another
3. This change of forms is mediated by some soil microorganisms
4. The inter-conversions help plants and other organisms in their nourishments, depending on the situation

6.0 TUTOR-MARKED ASSIGNMENT

1. What do you think is the major importance of nitrogen cycle to man and his environment?
2. Enumerate the components of nitrogen cycle and explain at least three of them
3. Explain the various sources of nitrogen to soils

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MODULE 2

UNIT 1 PHOSPHORUS CYCLE

CONTENTS

1.0 INTRODUCTION

Phosphorus (P), like nitrogen, also occurs in soils in both organic and inorganic forms. It can be found as either dissolved, in soil solution in very minute quantities or associated with organic materials or soil minerals. The relative amounts of each P form vary significantly with soils. The total amount of P present in the fine-textured clayey soils, for example, can reach up to ten times greater than that of a coarse-textured sandy soil. Understanding P cycle can assist crop producers in making decisions relating to P management on their farms, both for profitability and environmental protection. Most plants are only about 0.2 % P by weight, yet this relatively small quantity is still very critical. Phosphorus is an essential component of adenosine triphosphate (ATP). The ATP is crucially involved in most of the biochemical processes that take place in plants and it enables them extract soil nutrients. It also plays an indispensable role in DNA formation and cell development. Its deficiency in soil can cause reduced flower development, delayed maturity, low seed quality and decreased crop yield. Excess P, on the other hand can, sometimes, be deleterious. For example, increased P levels in fresh water streams and lakes can cause algal blooms due to eutrophication. When the algae die, they decompose to cause oxygen depletion which can lead to the death of lots of aquatic plants and animals. Phosphorus cycle is the biogeochemical cycle which characterizes the transport and chemical transformations of P via the geosphere, biosphere and hydrosphere.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- understand the process phosphorus cycle
- appreciate the importance of phosphorus cycle
- understand the problems associated with poor phosphorus management

3.0 MAIN CONTENT

3.1 Forms of soil phosphorus

a. Organic P in soils

A large number of compounds make up the soil organic P pool the majority of which are of microbial origin. Organic P is in a very tightly held forms, and generally not available for plant uptake until the P is released via mineralisation process following the decomposition of organic materials. The mineralisation is carried out by microorganisms, and like N, the rate of P release is affected by such factors as soil moisture, pH, composition of the organic material and aeration.

The reverse process of mineralisation, known as immobilisation, refers to the "tying-up" of plant-available P by microorganisms and/or soil minerals by using the available P for their own nutritional needs. Microorganisms may compete with plants for P, if the organic materials being decomposed are high in C and low in N and P, as in case of wheat straw. Both *mineralisation and immobilisation* occur in soil concurrently. If the P content of the organic material is high, however, enough to cater for the microbial requirements, then mineralisation will reign as a dominant process.

b. Inorganic P in soils

Concentration of inorganic P (orthophosphates) in soil solution is always very small. Phosphorus, in its inorganic form, occurs mostly as aluminium (Al), iron (Fe) or calcium (Ca) compounds. The chemistry of soil P is very complex, with more than 200 possible P minerals forms being affected by a number of physical, chemical and biological factors. A soluble P from commercial fertiliser or mineralisation, for example, reacts with soil constituents to form very low solubility (low plant availability) P compounds. This series of reactions is commonly called sorption or fixation. Iron and Al compounds will fix P in acidic (pH < 6) soil conditions, whereas under alkaline (pH > 7) conditions, P is preferentially fixed by Ca and Mg compounds. Figure 13 depicts the sequential process by which plant-available P is fixed by the soil minerals.

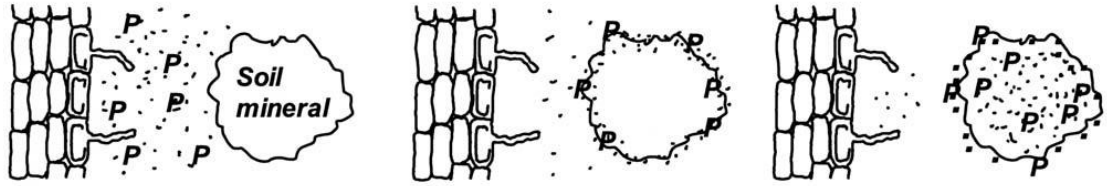

Figure 13. How phosphorus (phosphates) are tied up by soil minerals. A) A large percentage of the P is available for root uptake immediately after fertilization application. B) P in solution binds rapidly to the surface of soil minerals. Roots may still use this P. C) Eventually, most of the bound P becomes part of the structure of the mineral, with its plant availability being significantly reduced. Source: Espinoza et al. (2005).

Phosphorus availability to plants is, in most soils, greatest when soil pH is in the range of 6 to 7. Liming is a common production practice in raising the pH in acid soils so as to make P more available. Lowering the pH of calcareous soils, to increase P solubility is, however, not economically a viable option, as large amount of acidifying materials is required. Thus, soils with high pH generally have more P fertiliser applied needs. Soils without P fertilisation for a few years can fix much of the P applied as fertilizer, thereby making it unavailable. It is, therefore better to maintain proper P applications to soils and not mining out.

3.1.1 Phosphorus cycle

The human impact on the global P cycle has been substantial over the last 150 years. Because this anthropogenic modification began well before scientific efforts to quantify the cycle of P, we can only guess at the "pre-anthropogenic" mass balance of P. Several aspects of the P cycle are well-constrained (Figure 14). Phosphorus is initially solubilized, mainly

from apatite minerals, by chemical weathering during soil development. Physical weathering also plays a role by producing fine materials with extremely high surface area /mass ratios, which enhances chemical weathering in continental environments (i.e., floodplains, delta systems).

3.1.2 Phosphorus cycling in soils

The cycling of P in soils has received much attention, in terms of both fertilization and the natural development of ecosystems. Nearly 98 % of the about 122,600 Tg P within the soil and biota systems of the continents is held in soils in various forms. The exchange of P between biota and soils and is relatively fast, with an average residence time of 13 years. In soils, the average residence time of P is 600 years.

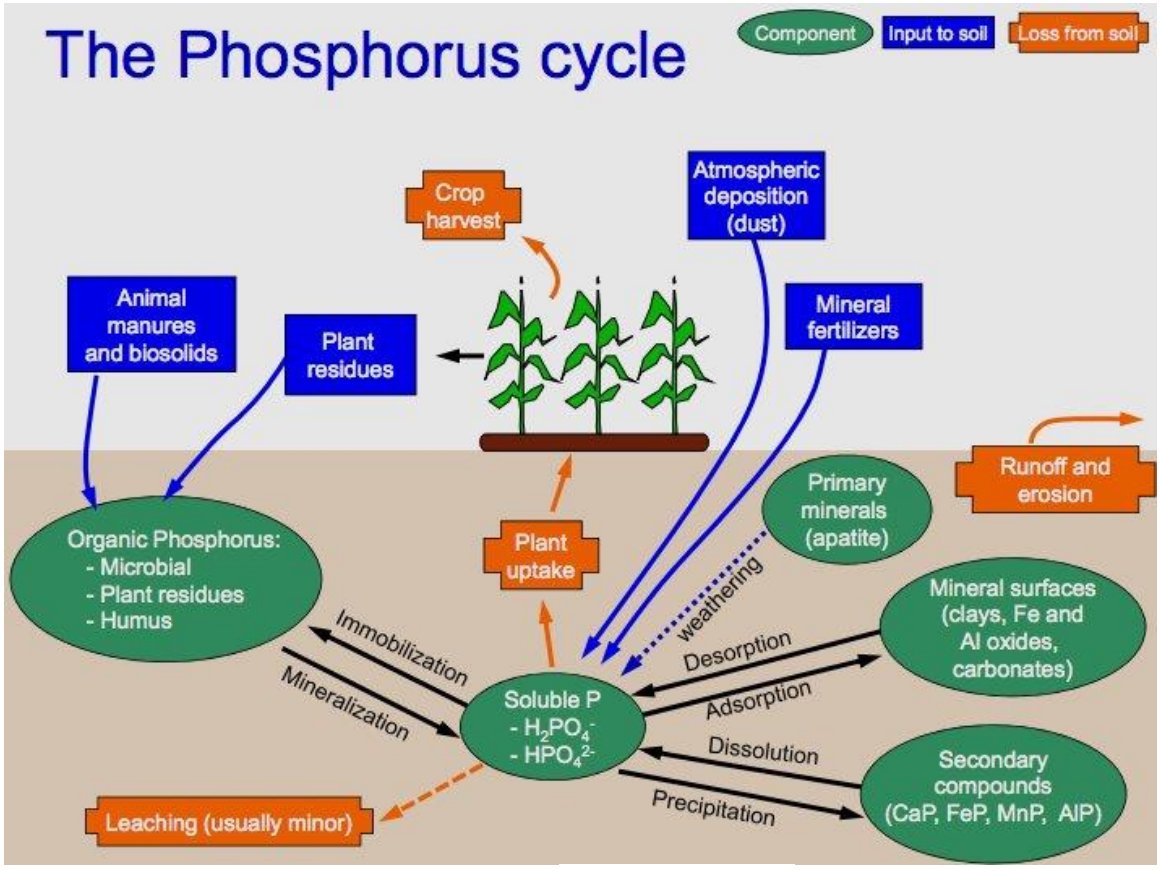

Figure 14. The phosphorus cycle in soils. Source: Turner et al. (2005).

The P cycle can be simplified as in Figure 15

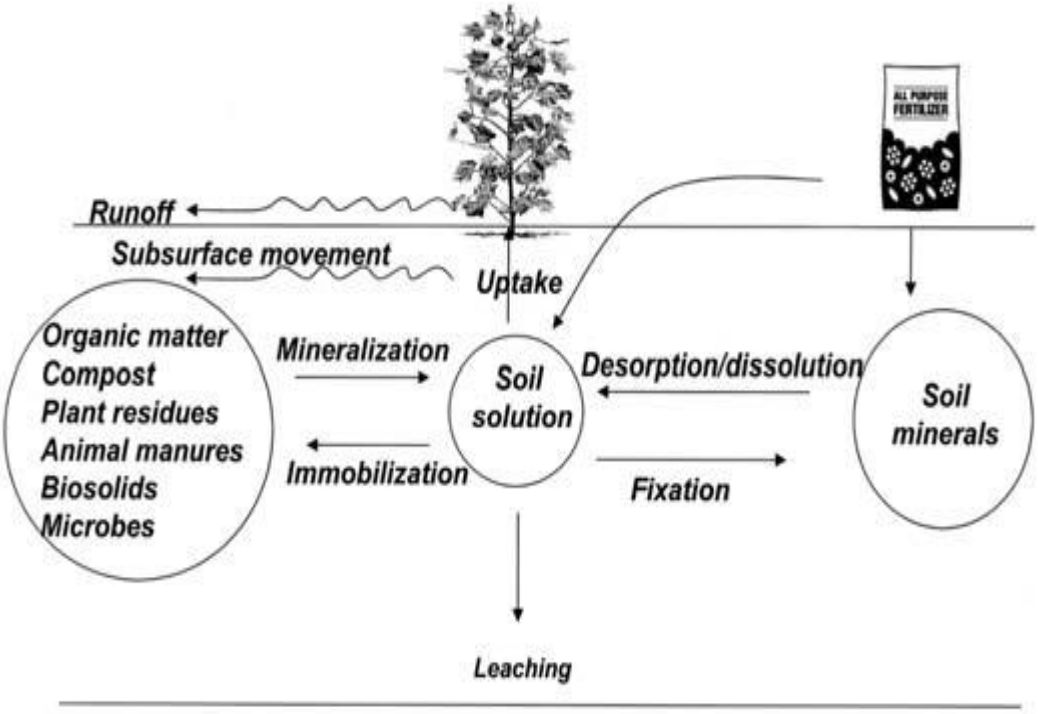

Figure 15. Simplified phosphorus cycle in soils. Source: Espinoza et al. (2005).

Phosphorus cycle, in Figures 14 and 15, shows these P forms and the pathways by which P may be taken up by plants or leave the site as P runoff or leaching. The general P transformation processes are: weathering and precipitation, mineralization and immobilization, and adsorption and desorption. Weathering, mineralization and desorption increase plant available P. Immobilization, precipitation and adsorption decrease plant available P.

3.2 Some roles of phosphorus

Role of phosphorus in biota

Phosphorus (P) is an essential plants and animals' nutrient ions form, including phosphate, PO_4^{3-} and hydrogen phosphate, HPO_4^{2-}. Plant species dissolve ionised phosphate forms and take the mineral into their system. Herbivores obtain their P by taking in plant biomass, and carnivores by consuming the herbivores. Both herbivores and carnivores excrete P as their waste product in faeces and urine. Phosphorus is then released back into soil when plants and/or animal matter decompose and the cycle repeats. Although phosphates are effective fertilizers, they are also aquatic pollutants. Because P is usually a limited supplied nutrient, even a slight increase in its availability can cause significant effects. Over-supplied phosphate condition can, therefore, lead to algae blooms. This excessive algae cause an increased consumption by bacteria, thereby leading to even higher bacterial populations. In the process, the bacteria use up much of the dissolved oxygen in the aquatic body for their cellular respiration which culminates in the death of fish due to suffocation.

The primary biological importance of phosphates is being a component of nucleotides, which serve as energy storage within cells, as Adenosine triphosphate (ATP) or, when linked together, form the two nucleic acids - deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Phosphorus, primarily in the form of hydroxyapatite, $Ca_5(PO_4)_3OH$, is a significant structural component of animals. About 80 % of the vertebrate animals' P is within their bones and teeth. This element is also an important constituent of phospholipids, which are in all biological membranes.

Anthropogenic influence

Human influences in P cycle arise mainly from the introduction of chemical fertilisers. Generally, use of fertilisers has significantly altered both the P and N cycles. Vegetation may not be able to utilize all of the phosphate fertilizer applied; as a repercussion, much of the fertilizer applied phosphate is lost from land via water surface runoff. The dissolved phosphate, in the surface runoff, is eventually precipitated as sediment at bottoms of water bodies. Animal wastes, or manure, are also applied to soils as fertiliser, especially in developing countries. Many other human sources of phosphate are in the out flows from municipal sewage treatment plants. The phosphate in sewage, without an expensive tertiary treatment, is not removed during various treatment operations. Again, through the process, an extra amount of phosphate enters the water bodies.

4.0 CONCLUSION

Phosphates move very quickly through the plants and animals; however, the cycling processes that move them through soils or ocean are very slow. This overall makes the phosphorus cycle one of the slowest biogeochemical cycles.

5.0 SUMMARY

In this unit, we have learnt that:

1. There is more than one type phosphorus inherently available in the soil
2. These P forms are cycled through the soil, water and living organisms

6.0 TUTOR-MARKED ASSIGNMENT

1. Explain the types of P available in the soil.
2. Write a full note on P cycle in soil.
3. Phosphorus: A possible cursed blessing. Discuss.

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UNIT 2 BIOLOGICAL NITROGEN FIXATION

CONTENTS

1.0 INTRODUCTION

Biological nitrogen fixation (BNF), also called biological dinitrogen (N_2) fixation, is the second most important biological process on earth after photosynthesis. It is the reduction of atmospheric N_2 to two molecules of ammonia. In the absence of modern fertiliser, or animal wastes, natural ecosystems rely on the biological conversion of the atmospheric N_2 to forms available for plant and microbial growth by a variety of prokaryotic microbes which are exclusive mediators. The NO_2-fixing microbes can exist as independent free-living organisms or in associations of differing degrees of complexity with other microbes, plants and animals.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- explain what biological nitrogen fixation is
- explain the significance of nitrogen fixation
- explain the biochemistry of the nitrogenase complex
- enumerate and explain, at least, two methods used in quantifying BNF

3.0 MAIN CONTENT

3.1 Significance of biological nitrogen fixation

Plants require relatively high level of N to produce abundant biomass or yield. All life forms require N to synthesise proteins and other important biochemicals, and N is often the limiting nutrient for plant and microbial growth in soils. Nitrogen for plant growth in natural systems comes from the soil, rainfall or other atmospheric deposition through BNF.

Industrial N_2-fixation amounts to 85 million metric tonnes per year and requires substantial inputs of energy usually in form of natural gas. This output is however, less than the values range of 100 to 180 million metric tonnes per year estimated contribution of BNF. Biological processes contribute 65 % of N used in agriculture, much of which is through symbiotic N_2-fixation. Non-symbiotic and associative N_2-fixations are of some significance in such crops as sugarcane and sorghum which has a C4 photosynthetic pathway and in specific ecosystem where N for growth of plant is a limiting factor. Flooded rice, due to its culture in flooded soils derives significant benefit from the activities of free-living diazotrophic bacteria and cyanobacteria. Biological N_2 fixation is an alternative to the use of expensive NH_3-based fertiliser N.

3.1.2 Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is the process whereby gaseous (atmospheric) nitrogen (N_2) is reduced to ammonia (NH_3) in the presence of nitrogenase. Nitrogenase is a biological catalyst that is naturally found only in certain microorganisms such as the symbiotic *Rhizobium* and *Frankia,* or the free-living *Azotobacter*. Biological NF is the major source of N input into agricultural systems. Rhizobia (a nitrogen-fixing bacteria), are symbiotic bacteria that elicit on the roots of specific legume hosts the formation of new organs (nodules), within which the bacteria proliferate, differentiate into bacteroids and subsequently fix N_2 into NH_3. The BNF is brought about by both free living soil microorganisms and symbiotic associations of microorganisms with higher plants. The *Rhizobia* infect root hairs of the leguminous plants and produce the nodules. Those nodules become the home for bacteria where they obtain their energy from the host plant and take free nitrogen from the soil air and process it into combined nitrogen. In return, however, the plant receives the fixed N from nodules and produces food and forage protein. Therefore, BNF is an efficient source of N.

i. The nitrogenase enzymes complex

So far, the BNF is restricted to prokaryotic microbes including many genera of soil bacteria, cyanobacteria (formally the "blue-green algae") and a few *Actinomycetes* (most notably *Frankia*). These microbes are capable of fixing dinitrogen as free-living forms (*that is,* not plant-associated), in loose association with higher plant, referred to as associative symbiosis, (*example.* many grass-bacteria associations) and in truly symbiotic partnership with higher plant (the root-nodule symbiosis involving *rhizobia* and *Frankia*) A trait shared by all of these symbiotic microbes is the production of enzyme complex called nitrogenase, which mediates BNF. Most appropriately, it is called the "nitrogenase complex" because it is composed of 2 protein components each composing of multiple sub-units (Figure 16). The nomenclature of the complex is, however, as follows:

1. The overall complex is known as nitrogenase;
2. Iron-molybdenum (FeMo) protein is dinitrogenase; and
3. The iron (Fe) protein is designated dinitrogenase reductase which functions in the reduction of dinitrogenase.

Below is the diagrammatic representation of the nitrogenase complex as shown in (Figure 16).

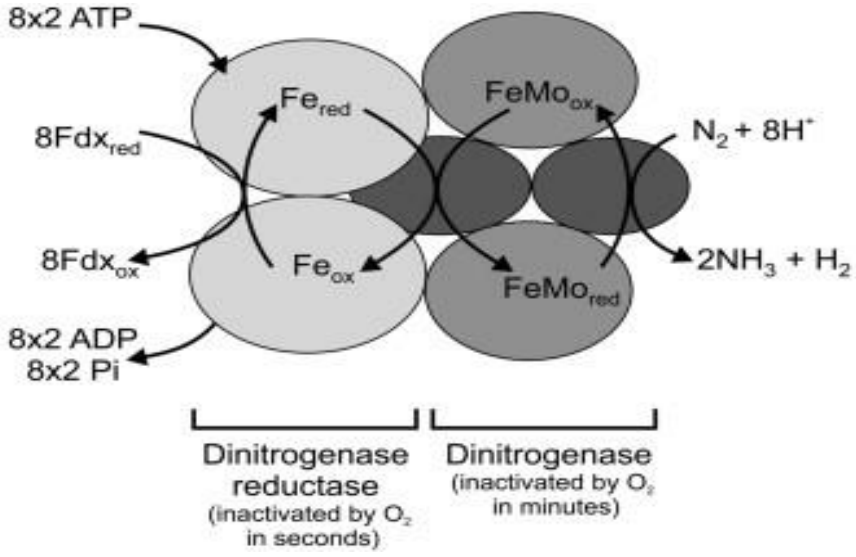

Figure 16. Diagrammatic representation of the nitrogenase complex. Source: Rascio and La Rocca (2008).

The overall reaction is as follows

$N_2 + 8H^+ + 8e^- + 16Mg\ ATP \longrightarrow 2NH_3 + H_2 + 16Mg\ ADP + 16Pi$

The mechanism can be summarised as thus:

1. The Fe protein accepts electron from a low-redox donor such as reduced ferredoxins (Fd_{red}) or flavodoxin and it binds two Mg ATP.
2. It transfers electrons one at a time to FeMo protein
3. The Fe protein and FeMo form a complex, the electron is transfer and two Mg ATP are hydrolysed to two Mg ADP + Pi (Phosphate)
4. Fe protein and FeMo dissociate and the process repeated.
5. When FeMo protein collects enough electrons, it binds a molecule of dinitrogen, reduces it and releases NH_4^+
6. The FeMo protein then accepts additional electrons from the Fe protein to repeat the cycle.

3.1.3 Methods of measuring N_2 fixation

Early studies of BNF were limited by the lack of techniques sensitive enough to measure N gains in plants or to measure the activity of the enzyme complex itself. Routine methods for measuring N_2-fixation or nitrogenase activity are, however, now available such as:

1. Nitrogen difference method

This involves the estimation of N_2 fixation by comparing the yields and N contents of plants grown with and without N_2-fixing bacteria. For example, comparing the yields of nodulated and non-nodulated legumes grown under similar conditions of soil N. The N in the non-fixing plant is the measure of the N acquired from soil and is subtracted from the N of the fixing plant, the difference is attributed to N_2 fixation.

2. Acetylene reduction assay (ARA)

The ability of the nitrogenase complex to reduce acetylene (C_2H_2) to ethylene (C_2H_4) forms the basis for the ARA. This is a gas chromatography based sensitive method that allows the detection of very low levels of nitrogenase activities. The sensitivity of the ARA facilitated attempts to measure N_2 fixation by grasses. The said conversion of acetylene to ethylene occurs by the following reaction:

$$C_2H_2 + 2H^+ + 2e^- \longrightarrow C_2H_4$$

In the ARA, the system to be measured, such as whole plants, isolated roots, soil cores or bacteria cultures, is exposed to an atmosphere containing 11 % acetylene incubated under appropriate conditions. Samples of the gas phase are periodically removed and injected into the gas chromatograph for quantification of ethylene production from acetylene.

3. Stable isotopes (^{15}N) method

These methods of BNF measurement make use of the stable, heavy isotopes ^{15}N and require a mass spectrometer (MS). High cost of the ^{15}N-labelled N sources, cost of analysis and, to some extent, availability of equipment or instrument are the major limitation of the methods.

The principal methods involved in studies using ^{15}N to measure N_2 fixation are:

i. Measurement of the incorporation of $^{15}N_2$ (labelled dinitrogen) into plant or microbial cells.
ii. Isotope dilution methods: in which the ^{15}N content in plant tissue is measured and the ratio of ^{15}N and ^{14}N are calculated and
iii. Natural ^{15}N abundance in soils or plants.

The ^{15}N natural abundance method is based on the fact that natural materials contain N that is naturally enriched with ^{15}N because of isotope discrimination during biological

transformations of N in soils. Therefore, plants containing less ^{15}N than the natural abundance can be suspected of supporting N_2 fixation.

4.0 CONCLUSION

Biological N fixation, carried out by rhizobia, leads to the reduction of molecular nitrogen (N_2) to ammonia (NH_3), subsequently assimilated in amino acids, and provides the Earth's ecosystems with about 200 million tonnes of N per year.

5.0 SUMMARY

In this unit, we have learnt that:

1. Biological N fixation can be used as a good N source for crops
2. The nitrogenase enzyme is important in BNF
3. There are a number of mechanisms before nitrogenase enzyme works

6.0 TUTOR-MARKED ASSIGNMENT

1. Describe the mechanisms involved in the nitrogenase complex
2. Explain the difference between nitrogenase reductase and nitrogenase
3. In your opinion, which of the methods of BNF quantification is the best? Why do you think so?

7.0 REFERENCES/FURTHER READING

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UNIT 3 MYCORRHIZA

CONTENTS

1.0 INTRODUCTION

There are many types of mycorrhizal associations, of which the endomycorrhizal association of the vesicular arbuscular (VA) type are geographically the most widespread, as well as within the plant kingdom. Vesicular arbuscular mycorrhizal fungi (VAM) invade cortical cells inter- and intra-cellularly and form clusters of finely divided hyphae known as arbuscules in the cortex. They also form membrane-bound organelles of varying shapes known as vesicles inside and outside the cortical cells**.**

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- describe what mycorrhizas are
- identify the diversity and role of mycorrhizas

3.0 MAIN CONTENT

3.1 Mycorrhiza

The term “mycorrhiza”, as coined by Frank, A.B. a German researcher more than 100 years ago, means “fungus-root”. It stands for the mutualistic association existing between higher plants and a group of soil fungi.

3.1.1 Types of mycorrhizas

There are two main types of mycorrhizas, ecto and endomycorrhizas, which differ in their structure and physiological relationships with host plant. Mycorrhizal fungi frequently stimulate plants to reduce root biomass while simultaneously expanding nutrient uptake capacity by extending far beyond root surfaces and proliferating in soil pores that are too small for root hairs to enter. Legume roots are invaded and colonised by rhizobia and also with mycorrhizal fungi. For legumes, arbuscular mycorrhizal (AM) fungi have fundamental effects on the eco-physiology, on the biota of the surrounding soil and on associated non-legumes. Arbuscular M-fungi are known to be effective in increasing nutrient uptake, particularly phosphorus and biomass accumulation of many crops in low phosphorus soil. AM-fungi have an important role in promotion of biological and chemical properties of plants under stressed environment. Most reports note a positive effect of mycorrhizal inoculation on growth of plants in metal-contaminated soils.

There are various mycorrhizal morphologies, depending on the fungal and plant types involved in the symbiosis, for example:

- Vesicular-Arbuscular (VA) mycorrhizas: Fungi: Glomalean (130 spp.), Plant: Gymnosperms, Angiosperms, Pteridophtyes, Bryophytes, generally have low host specificity
- Ectomycorrhizas (ECM): Fungi: Basidiomycete and Ascomycete (6000 spp.), Plant: Gymnosperms and Angiosperms, low to moderate host specificity
  - Arbutoid mycorrhizas: Fungi: Basidiomycete, Plant: Ericales
  - Ericoid mycorrhizas (ERM): Fungi: Ascomycete, Plant: Ericales and Byrophytes
- Monotropoid mycorrhizas: Fungi: Basidiomycete, Plant: Monotropaceae (Ericales), high fungal specificity
  - Orchid mycorrhizas: Fungi: Basidiornycete, Plant: Orchidaceae

Although the mycorrhizal structural forms vary considerably, their main functions are similar as summarised below.

3.2 Occurrence of mycorrhizas in nature

a. Up to 90 % of all land plants belongs commonly to mycorrhizal families
   - Mycorrhizas have been described in 83 – 85 % of dicots, 79 % of monocots, and most conifers (9 5% of Pinaceae)
   - They are easier to identify than non-mycorrhizal families, typically dominated by ruderal species (*Brassiacaceae*) or having different root structures (*Proteaceae*)

b. Widely distributed across nearly all major biomes:
   - VA typically dominates in grasslands, shrub lands, tropical rainforests, typically areas with P limitation, - 80 % of all plant species are VA.
   - ECM dominate in temperate forests, particularly coniferous forests, typically areas with N limitation, lower % of plant species, but high % of land coverage.
   - ERM dominates in healthy lands, alpine environments.

c. The mycorrhizal symbiosis has a *long* history (V A - 500 mya; ECM - 130 mya) and was probably key in allowing plants to colonise land.

3. Functional aspects of the mycorrhizal symbiosis

The mycorrhizal symbiosis is a trading system in that, fungi have extensive mycelial networks for water and nutrient uptake, but need a C source. Plants, on the other hand, have an autogenic C source, but need to have water and nutrients from the soil, therefore, plants give the fungi C, typically as a sugar, and fungi pass nutrients to plant. Mycorrhizal fungi have other functional attributes aside from facilitating water and nutrient uptake, such as protecting plants from soil heavy metal concentrations and root pathogens.

4. Mycorrhizas and plant nutrition

Typically, mycorrhizal plants have much higher nutrient contents than the non-mycorrhizal plants. Root system extension allows mycorrhizal plants to access larger soil area. Fungal hyphae also have smaller diameters than roots and can, therefore, access smaller soil pores, which effectively increases the soil volume exploited, a good evidence for this mechanism. Fungi may be more effective nutrient competitors against free-living soil microorganisms than roots, the evidence for this mechanism is not

conclusive, or fungi may alter the rhizosphere bacterial community in ways that facilitate nutrient capture, the evidence is small but possible. They may also have different uptake kinetics than roots, leading to more effective uptake at lower nutrient concentrations. Fungi also have much higher surface-to-volume ratio than roots, which may actually increase the rate at which nutrients are absorbed. Fungi also enzymes, such as phosphatases and proteases, and chelating compounds capable of capturing nutrients from soil organic and inorganic materials that are not normally accessible to plants.

5. Mycorrhizas and plant water relations

Mycorrhizal fungi can alter the plant-water relations in some ways, which result in an increased water capturing.

Plant size:

- The mycorrhizal plants root systems are usually larger and more finely divided than the non-mycorrhizal plants.

Unrelated to plant size:

- Nutritional: P starvation can affect stomatal conductance.
- Physiological: Transpiration rates can also be altered by mycorrhizal fungi
- Environmental: Soil structure can be altered by mycorrhizal fungi in ways that increase soil water content.

6. Mycorrhizas and plant growth

There is conflicting evidence on the effects of mycorrhizas on plant growth. Many studies have shown positive growth whereas others showed neutral or negative growth.

It is, however, important to note that:

- Most of mycorrhizas and plant growth studies have been done under laboratory conditions in which growth effects may be affected by some other factors.
- The plant growth stage may also affect whether the growth is positive or negative. Seedlings with small C quantities may be negatively affected by rnycorrhizas, whereas larger plants may be affected positively due to a more positive C balance.
- Although growth is usually correlated with fitness, and therefore used in many studies to indicate the importance of a certain factor, survival is paramount to fitness, and by amending nutrient and water conditions, mycorrhizal effects may have a greater impact on survival than on growth.

7. **Mycorrhizas and plant ecology**

Aside their role in water relations and plant nutrition water relations, mycorrhizal fungi can have major ecological impacts on:

- Plant Establishment and Succession
- Plant Community Diversity
- Ecosystem Biogeochemistry

8. Cost of the mycorrhizal symbiosis

- Range of Estimates:

About 4 - 20 % of total plant C is transferred to the fungus, multiple studies using independent methods have estimated -15 %.

- Environmental conditions have a large impact on whether the symbiosis has a positive or negative outcome.

3.3 Arbuscular mycorrhizal fungi

Arbuscules are believed to be the sites for exchange of materials between the host and the plant. Generally, vesicles serve as mere storage structures, and when old they could serve as reproductive structures. Vesicles and arbuscules together with large spores form the diagnostic feature of the VAM associations as in Figure 17. Vesicles are absent in two of the seven genera containing these fungi, due to this, therefore, the term arbuscular-mycorrhizal (AM) fungi is currently preferred by most researchers in order to represent the association rather than vesicular-arbuscular (VA) mycorrhizal fungi. Arbuscular mycorrhizal fungi occur on a wide spectrum of temperate and tropical plant species and are absent in less than 30 plant families

Arbuscular mycorrhizal development

The establishment of AM symbiosis can be visualised as a programmed sequence of phenotypic changes, corresponding to distinct recognition events which lead the two partners, host plant and fungal symbiont, to a high degree of morphological and physiological integration. Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs, unable to complete their life cycle during asymbiosis (Bonfante and Bianciotto, 1995). Arbuscular mycorrhizal fungal spores are the only plant-independent phase of the mycobiont. They are round-shaped structures with a thick cell wall and average diameters between 50 and 100 μm. They contain a very large number of nuclei, of up to 2000 per spore (Becard and Pfeffer, 1993). After germination, hyphae are always coenocytic. Studies on two AM fungal species have shown that these are haploids with an unusually high genetic variation (Hijri and Sanders, 2004; Hosny et al., 1997).

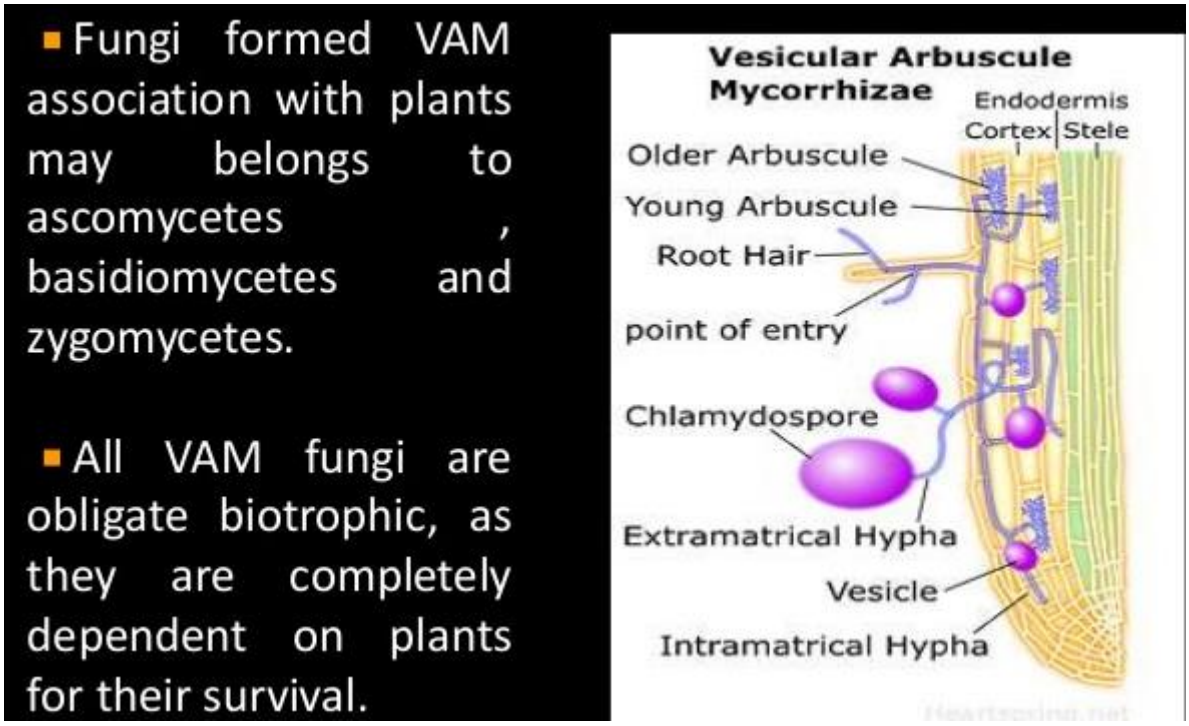

Figure 17: The VAM Structure with spores

Arbuscular mycorrhizal life cycle

The establishment of the AM symbiosis begins with the colonization of a compatible root by the hyphae produced by AM fungal soil propagules, asexual spores or mycorrhizal roots. After attachment of a hypha to the root surface by means of an aspersorium, the fungus penetrates into the cortex and forms distinct morphologically specialized structures: inter- and intracellular hyphae, coils and arbuscules. Arbuscules are specialized hyphae, similar to haustoria from the plant pathogenic fungi, formed as intercalary structures between the coil hyphae and are the site of mineral nutrient transfer to the plant and potentially the site of carbon acquisition by the fungus. After host colonization, the fungal mycelium grows out of the root exploring the soil in search of mineral nutrients, and it can

colonize other susceptible roots. The fungal life cycle is completed after formation of asexual chlamydospores on the external mycelium. Distinct morphological stages can therefore be identified during the life cycle of arbuscular mycorrhizal fungi. This clearly shows that the host plant plays a key role in orchestrating the AM infection process. The sequence of steps leading to an AM symbiosis is largely conserved among different combinations of fungal and plant species. Overall, these developmental processes require molecular communication between the AM fungus and the plant, including exchange and perception of signals by the symbiotic partners. Thus, the complex morphological and physiological alterations of both symbiotic partners accompanied by the recognition process suggest that AM symbiosis is the result of multifaceted, fine-tuned signalling events.

3.4 Mycorrhizae and their diverse roles

Mycorrhizae are the rule in nature, not the exceptions. In this association, the fungus takes over the role of the plant's root hair and acts as an extension of the root system. The beneficial effects of AMF result from one or several of the mechanisms. With mycorrhizal colonisation in the roots, there is increased absorption surface area, greater soil area exposed greater longevity of absorbing roots, better utilisation of low-availability nutrients and better retention of soluble nutrients. This reduces reaction with soil colloids or leaching losses. Arbuscular mycorrhiza increase establishment, nodulation and N_2 fixation capacity in legumes. Mycorrhizae influence the colonisation of roots by other microorganisms and reduce the susceptibility of roots to such soil-borne pathogens as nematodes and/or phytopathogenic fungi. They also modify soil-plant-water relations, thus promoting better adaptation of plants to such adverse conditions as drought, salinity or heat. At elevated heavy metal concentrations in soils, AMF have been shown to detoxify the environment for plant growth. In a nutshell, therefore, mycorrhizae connect primary producers (plants) to the heterogeneously distributed nutrients required for their growth. Hence, understanding the ecology and functions of AM symbiosis, in the natural or agricultural ecosystem, is essential in improving plant growth and productivity.

4.0 CONCLUSION

Mycorrhizal symbiosis is one of the crucial factors that determine plant and soil health. In addition, mycorrhiza enhances mineral uptake ability and drought stress tolerance. It also induces resistance against soil pathogens, and reduces sensitivity to toxic substances in their host plants. The present day agricultural practices may, however, lead to the destruction of these beneficial associations.

5.0 SUMMARY

1. Mycorrhiza is a mutualistic relationship between fungi and plant root.
2. There are diverse types of mycorrhiza in existence.
3. They also play diverse and crucial roles in the soil environment.

6.0 TUTOR-MARKED ASSIGNMENT

1. Mycorrhizas can be said to be an indispensable force to reckon with in terms of plant and general soil health. Discuss this assertion with cogent reasons.

7.0 REFERENCES/FURTHER READING

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UNIT 4 ORGANIC MATTER

CONTENTS

1.0 INTRODUCTION

The mineral soils surface contains an accumulation of living biomass, dead and decomposing organic material and humus. Typically, this soil organic matter (SOM) accounts for 1 – 10 % of the total soil mass. It is, however, difficult to be isolated from the soil because of its intimate association with the soil mineral fraction. The larger, recognisable remains of animal, plant and soil organisms that can be separated from soils by hand picking and sieving techniques are called particulate organic matter. These tissues undergo continuous decay, and over periods of years to decades, brown to black-coloured, colloidal humus is synthesized and accumulates. Soil humic substances can account for 50 – 60 % of the total SOM, and together with the non-humic material provide a nutrient reservoir to sustain the soil microbial biomass.

2.0 OBJECTIVES

At the end of this unit, you should be able to:

- understand what organic matter is
- appreciate the various sources of organic matter
- describe the functions of organic matter

3.0 MAIN CONTENT

3.1 Organic matter

Organic matter, also termed as organic material or natural organic matter (NOM) refers to the large pool of C-based compounds found within natural and anthropogenic, terrestrial and aquatic environments. Organic matter in soil contributes to the soil productivity in many different ways It is a matter that is composed of organic compounds that come from the remains of such organisms as plants and animals and their waste products in our environment. Organic molecules can also be made by chemical reactions devoid of life. Basic structures are created from cellulose, cutin, tannin and lignin, along with other various proteins, lipids and carbohydrates. Organic matter is very important in nutrients' movement in the environment and plays a significant role in water retention on the surface of the planet.

3.1.1 Organic matter contents

Organic matter is mainly present in the top 20–30 cm of most soil profiles and is essentially an array of organic macromolecules that consist, principally, of combinations of C, H, O, N, P and S. Soil organic matter is commonly measured as the quantity of organic C. Almost all organic matter in soil is derived, directly and indirectly, from plants via photosynthesis. Thus, atmospheric CO_2 is transformed into simple and complex organic C compounds by reduction which, in combination with key nutrients, enable the plant functioning and growth. Carbon dioxide is directly released from plants through respiration, but most of the fixed C is retained and ultimately transferred into the soil ecosystem via a combination of spatially distinct pathways over a variety periods.

The most important pathways are exudation of soluble organic compounds from roots, direct addition of senescent material as above-ground and below-ground detritus and return of ingested plant matter in animal faeces.

3.1.2 Organic matter source of energy and nutrients

Root exudates and plant and animal detritus represent the essential energy and nutrient sources for soil microbial and faunal communities. Bacteria and fungi represent over 95 % of the biomass present in most soils. They interact with combinations of micro-fauna, such as nematodes and protozoa; meso-fauna, such as acari, Collembola and mites; and such macro-fauna as earthworms, termites and molluscs, in complex soil food-web systems that direct the organic matter and associated nutrients' turnover in the soil environment. Organic C decomposition in soil is, primarily, driven by bacterial and fungal activities, whereas only 10 – 15 % of soil C flux can be attributed, directly, to faunal actions.

The majority of soil microbes are heterotrophs that rely on organic matter for nutrients and energy. These can be mainly divided into microbes that primarily respond to addition of fresh C substrates (*i.e.* zymogenous or *r*-selected biomass) and those that derive energy mainly from the decomposition of older, more recalcitrant forms of organic C (*i.e.* autochthonous or *K*-selected biomass).

Root exudates

Soluble plant root exudates account for a range of 10 – 40 % of the total C that is fixed by photosynthesis and are mainly composed of mixtures of amino acids, sugars, organic acids, sugar alcohols and secondary metabolites. Root exudates are particularly important drivers of faunal and microbial activity in soil due to a combination of their high bioavailability relative to the senescent plant debris, the role they play in controlling bioavailability of nutrients (*e.g.*, P) and phytotoxic elements (*e.g.*, Al), together with the fact that they are added to soils on a regular to semi-continuous basis. Thus, numbers and activity of microbes in the closest vicinity of growing roots (*i.e.*, 1 – 3 mm, the rhizosphere) are normally greater, orders of magnitude, than in non-rhizosphere soil (*i.e.*, bulk or edaphosphere). Ratios of microbial communities in rhizosphere and adjacent edaphosphere soil vary with environmental conditions and plant species, although 12 – 25-fold differences in bacterial and fungal populations have been observed by some scientists. Plants, therefore, directly benefit from an exudate enhanced biological activity in the rhizosphere, majorly via an improved acquisition of sparingly soluble and organic soil nutrients mobilised by microbes in response to the provision of energy-rich C substrate.

Organic matter contained in living biomass is the most reduced material in the biosphere, and it ranges, in soils, from total dominance, as in peatlands, to the minor amounts found in young soils or at depth in vadose zone. Soil organisms generate electrons during the metabolic oxidation of organic matter, and these electrons must be transferred to an electron acceptor, the largest of which is atmospheric O_2 in freely drained, aerobic soils. The O_2 trapped in the soil or present in the water can be consumed within hours by soil microbes and is replenished by O_2 diffusion.

3.1.3 Factors controlling the decomposition of organic materials

The factors that control the decomposition of organic materials are determined by three sets of interacting factors as thus: i. substrate quality, ii. organisms and iii. environment. Thus, decomposition does not occur at uniform rate in either time or space. In the initial decomposition phase, which typically lasts for less a year, the majority of such readily metabolised components as unprotected sugars and oligosaccharides, proteins and amino acids, are rapidly exploited by the decomposer organisms leaving more recalcitrant components that may resist breakdown due to their biochemistry or by physical protection within the soil matrix. The long-held view that lignin components and its derivatives are inherently more stable than other components of plant materials is now being questioned. Even simple organic substrate components may become stabilised for millenia in the 'passive' organic matter pool by interacting with soil mineral colloids, encapsulating in soil aggregates where the local environmental conditions preclude rapid decomposition (e.g. due to limited O_2 diffusion), and interaction between organic compounds which leads to an increased chemical complexity that is resistant to microbial decomposition.

A usually-bewildering array of methods has been developed to define different soil organic matter pools. These include separation of living from non-living organic matter components, which can then be further divided into more specific fractions, based on chemical (alkali-acid solubility), physical (size fractions) and kinetic functional (*i.e.,* susceptibility to decomposition) variables as depicted in Figure 18. The non-living soil organic matter can, on the other hand, be divided into some fractions, based on a combination of chemical form and physical size. Plant and faunal detritus, identifiable, ranging from over 2 mm down to 50 mm in size can be physically separated from soil by sieving and density floatation, although these fractions are mostly not considered as a part of soil organic matter. The remainder of the non-living soil organic matter is comprised of organic macromolecules, known collectively as 'humus'. Humus can further be divided into two broad categories of compound, as: non-humic (ca. 30 %) and humic (ca. 70 %) substances. Non-humic substances are defined as chemically identifiable plant, microbial and faunal constituents, including nucleic acids, peptides and amino acids, sugars and polysaccharides, lipids and lignin.

3.2 Benefits of stable soil organic matter

There are many benefits to have a relatively high a level stable organic matter in agricultural soils. The benefits can be grouped into three main categories, as follows:

a. Physical benefits

- Enhancement of aggregate stability, thereby improving water infiltration and soil aeration and reducing surface runoff.
- Improvements in soil water holding capacity.
- Reduction in the level of stickiness in clay soils, thereby making them easier to till.
- Reduction in soil surface crusting, which facilitates easier seedbed preparation.

b. Chemical benefits

- Increases the CEC of soils and/or its capability of holding onto and supplying, over time, such essential nutrients as Ca, Mg and K.
- Ensures improvements in buffering capacity of soil, which is its ability to resist a sudden change in pH.
- Accelerates the decomposition of soil minerals over time, thereby making them in their minerals and available for plant uptake forms.

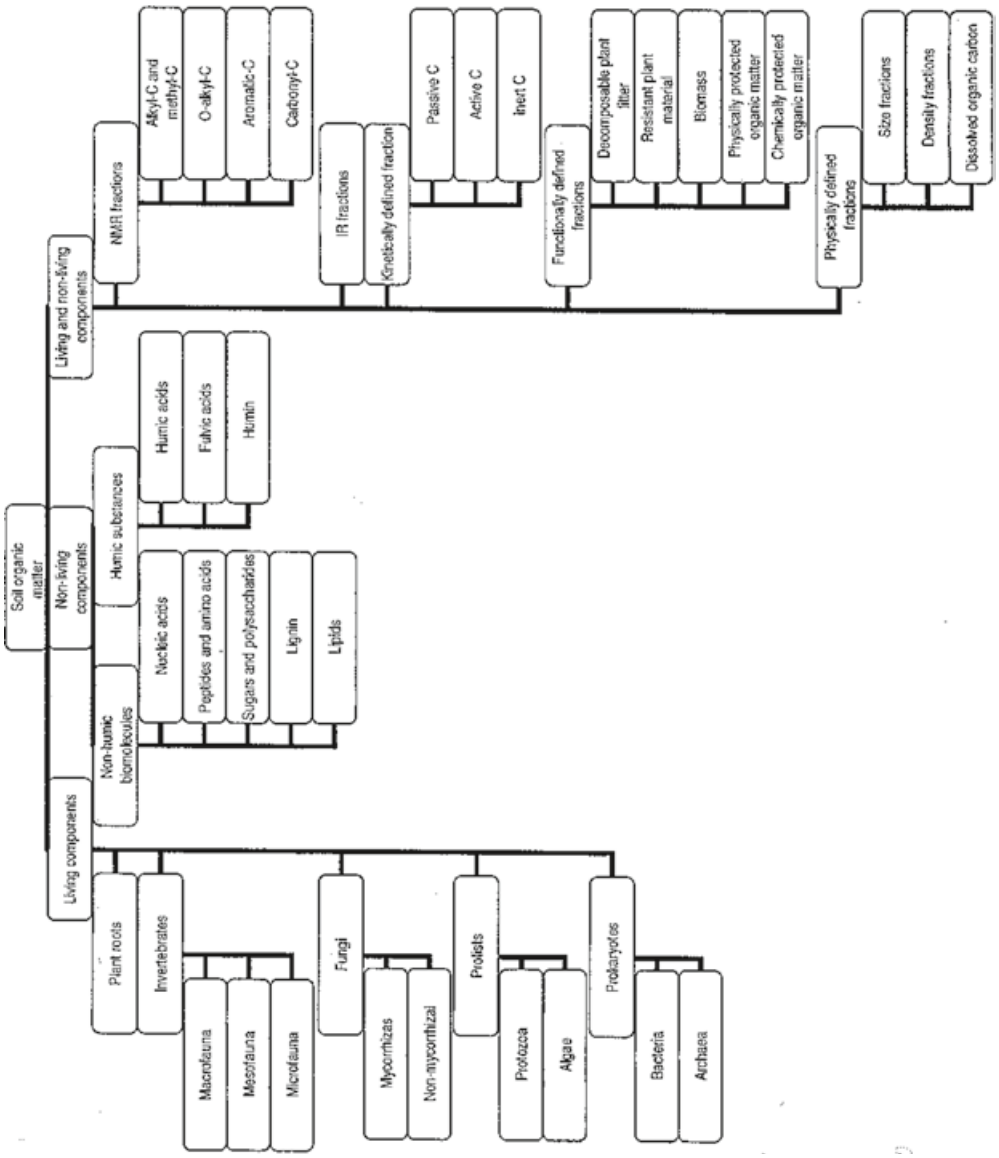

Figure 18. Summary of soil organic matter fractions (in: Bardgett, R.D., Usher, M.B., and Hopkins, D.W. (2005) (Eds.). Biological Diversity and Function in Soils; Hopkins, D.W. and Gregorich, E.G. Carbon as substrate for soil organisms. Cambridge University Press.

c. Biological benefits

- Provision of food for the living soil organisms.
- Enhancement of soil microbial activity and biodiversity, which can assist in pests and diseases suppression.
- Enhancement of soil pore spaces via actions of soil microorganisms. This assist in an increased infiltration red and reduced soil surface runoff.

Generally, application and incorporation of organic materials can, over time, result in an overall increased stability of organic matter levels in soil.

4.0 CONCLUSION

Organic matter is commonly available in the ecosystem and is cycled via decomposition processes undertaken by soil microbial communities that are crucial for availability of nutrients. It can move into soil and mainstream water via water flow after degradation and reactions. Organic matter provides means of nutrition to living organisms. It also acts as a buffer in aqueous solution in order to maintain a neutral pH in the environment. The buffering component has been proposed to be very relevant in neutralising acid rains. With careful management, therefore, the preservation and accumulation of soil organic matter can

readily see to the improvement of soil productivity which can result in greater farm productivity and profitability.

5.0 SUMMARY

In this unit, we have learnt that:

1. Organic matter is composed of many components.
2. Organic matter has many physical, chemical and biological advantages for soil and soil and other organisms.

6.0 TUTOR-MARKED ASSIGNMENT

1. Write short notes on the physical, chemical and biological advantages of organic matter.
2. Organic matter can be said to be the precursor to a healthy soil. Briefly discuss on this statement.

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UNIT 5 FATE OF CROP RESIDUES

CONTENTS

1.0 INTRODUCTION

The term crop residues refer to such parts of crop plant that are not considered the main economic produce of the crop which are left behind after the economic part has been harvested or removed. For example, rice crop, *Oriza* spp. that is cultivated primarily for its grain yield has its straw, leaves and roots as the residue. Forest trees and surviving crops do not form part of crop residues. Crop residue forms a considerable fraction of natural organic material, and has a large pool of carbon – based compound. Chemically, crop residues composed majorly of complex compounds like cellulose, lignin, cutin and minute fraction of protein, lipid and carbohydrate. Over the years, the significance of crop leftover in the control of soil erosion and fertility and structural improvement of soil has been well recognized globally. However, a number of uses of these secondary products of crops such as animal feed, building material, matting, source of fuel and others have drastically reduced its application for soil conservation, fertility and crop productivity.

2.0 Objectives:

At the end of this unit, students should be able to:

- Comprehensively define crop residue
- Mention and describe various categories of crop residues
- Explain possible fates of crop residue
- Explain decomposition process of crop residue

3.0 MAIN CONTENT

3.1 Categories of Crop Residues

We have the following categories of crop residues:

1. Forage legume residues: This comprises of the root of *Alfalfa spp , Centrocema spp, Lablab spp, Mucuna spp.*
2. Forage grass residues: This comprises of the roots of guinea grass, *Digiteria spp*, elephant grass.
3. Legumes crops residues: This comprises of haulms and roots of groundnut, cowpea, bambaranut, sunflower.
4. Cereal crop residue: This comprises of the stalk, roots, leaves, bran and husk of rice, millet, maize, wheat, sorghum

3.2 Fate of crop residues

Crop residues are subject to various fates which can be broadly classified into in – situ and off – situ fate:

In-situ fate: This refers to all possible conditions that get subjected to crop residues in the farm. This includes

i. Burning

Burning has been a long traditional practice among local farmers especially in the tropics. Various purposes lead to the adoption of this practice. Farmers usually burn down heaped crop residue to ashes for farm clearance, control of pest and disease (e.g. rodents and bird pest), hunting of animals and for regeneration of fresh pasture needed to feed their livestock. Burning could also occur naturally due to bush fire. Generally, burning has negative effects on soil fertility and productivity as it increases the pH level of soil, kill decomposer microorganisms and depletion of important soil nutrients such as nitrogen, sulfur and phosphorus and potassium and carbon. The release of CO2 into the atmosphere due to burning pose serious threat to ozone layer which protect the earth from direct solar emission, thus, increase global warming effect.

ii. As animal feed

In tropical countries, farmers do allow their animals to feed upon crop residues in the farm. This kind of practice has the potentialities of enhancing soil productivity. This holds true, because the animals defecation and urine add nutrient to the soil and provide good thriving ground for the growth and proliferation of beneficial microorganism.

iii. Incorporation into the soil

This is usually done during pre-planting operation, specifically ploughing tillage operation. The residue is broken down into small pieces and buried into the soil. This help in improving the fertility status of the soil, enhance aggregation of soil particles and increase water and nutrient retention of the soil which translate to enhanced productivity and crop production profitability.

iv. **As mulching material and for bedding**

Some farmers use stalks and straws to cover soil surface for preservation of available soil water, heat generation to facilitate germination as well as to protect sown seed and newly germinated pant from attacks by pest.

Off – situ fate

This refers to all possible conditions that crop residues get subjected to outside the farm. This includes:

i. Use as building material

Another important off-site fate of crop residues is in its use for numerous building purposes such as thatch, fencing material, binding agent for moulding and block making.

ii. Use as source of fuel, matting, and in construction of bed

Domestically, rural farmers use crop residues for cooking and heating, knitting of mat and bed for storage and drying purpose.

3.3 Residue decomposition

Heterotrophic organisms inhabiting the soil break down larger crop residue by their activities. Upon decomposition, the heterotrophs use organic compound such as carbon, nitrogen a phosphorus and other nutrient for their energy and general metabolic activities. Biological crop residue decomposition lead to the release of large amount of plant nutrient in the process called mineralization. However, the process is very slow especially, in the dry savannah where the climatic variables are not very conducive for optimum microbial activities. Several factors dictate mineralisation rate and amount of nutrient release. These include soil moisture status, soil temperature, pH, quality of the residue, type and number of the microbes (*e.g.* C: N ration, presence of fibre). Upon decomposition, the crop residue is

transformed to minute amorphous substance called the humus. Most decomposition occurs near the soil surface, where plant litter inputs are concentrated.

4.0 CONCLUSION

The fate of crop residues can be categorized into two broad categories: the in-situ and the off-situ otherwise on-site and off-site. Incorporation of crop residue into the soil enhances soil fertility and productivity and also controls erosion tendencies.

5.0 SUMMARY

In this unit, we have learned that

1. Crop residue is any part of crop plant other than the main or primary part of concern of the production e.g. root, bran, straw of rice crop, leaves and stems of cassava crop
2. Various fates of crop residues include burning, incorporation into the soil, building and fuel material
3. Decomposition of crop residues releases nutrients for plant uptake and is facilitated by soil microorganism

6.0 TUTOR – MARKED ASSIGNMENT

1. What did you understand by the term 'crop residues?

2. Mention five fates possibly encountered by crop residues

3. Separately, explain the effect of crop residue burning and incorporation into the soil in soil conservation and crop production

4. What are the conditions necessary for mineralization?

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Module 3

UNIT 1 ANIMAL WASTES IN SOILS

CONTENTS

1.0 INTRODUCTION

Animal waste otherwise called animal by-product commonly refers to the excreted materials by live animals. However, in production systems, materials in degraded form found together with the excreted by-products are considered animal waste. The use of animal waste for agricultural crop production and soil health improvement is the key principle/ rationale behind mixed farming practice (a farming system in which crops and animal are managed on the same piece of land, the animals are allowed to feed on the crop residues after harvest while their waste improve soil quality and crop productivity). The estimated global annual production of animal waste stands at 16 – 20 MMT. Examples of animal waste comprised of urine, excreta, fur and hair, scales e.t.c

2.0 OBJECTIVES

At the end of this unit, students should be able to:

1. Define and give examples of animal waste
2. Explain sources of animal waste and its mode of diffusion
3. Highlight the importance of animal waste in relation to soil and crop production
4. Enumerate the negative effects of over-application of animal waste in crop production

3.0 MAIN CONTENT

3.1 Sources of Animal Wastes

Two sources of animal waste can be recognised

1. Primary source
2. Secondary source

1. **Primary source**

Animal waste directly excreted by live animal fall under this group. Animal waste around our environment largely comes from livestock - mostly large and small ruminant e.g. cattle, sheep and goat, non - ruminant e.g. swine, pseudo-ruminant e.g. rabbits and camel, birds e.g. poultry, guinea fowls, and fishes e.g. cat fish, tilapia.

2. **Secondary source**

This comes from processing industries such as ternary, meat processors, abattoirs, fish market *etc*.

3.1.1 Mode of diffusion of animal waste

Animal waste get diffused into the environment through range and pasture production, confined or concentrated system or by transportation by man. In range and pasture production system the animals are allowed to cover large area of land, thus, the waste is more dispersed compare to where the animals are confined to a smaller unit of land. Man transport animal waste product as manure in bulk to supply nutrient to his farm. The release of evacuated waste by-product of animal by agro-based industries into waterways and unoccupied land add more waste to the environment.

3.1.2 Agricultural value of animal waste

In traditional farming system, the application of animal waste as a nutrient source/fertilizer is not uncommon. In modern practice, integrated soil fertility management practice (ISFM) (is a set of management practice aimed at combining organic input, chemical fertilizers, improved germplasm and appropriate local practices to improve productivity gains and resilience and environmental safety) advocate the use of organic based nutrient source such as animal waste to boost production. It has been has reported that cattle, poultry and swine in China produced about 4.9 MMT of phosphorus contained in animal manure. Poultry manure and livestock and rabbit urine are the best source for nitrogen and phosphorus and potassium. Cow dung on the other hand contains high amount of cellulose and hemi-cellulose which provide good soil structure and enhance microbial growth.

3.1.3 Animal waste as a source of pollution

Mass application of animal waste creates unpleasant odour and is nuisance to sight. Animal waste contained several types of pollutants (Table 5) such as arsenic, sulphur, cadmium, nitrate.

Table 5: Animal wastes as potential harbour pollutants

Pollutant	Effect	Remark
Nitrate	Blooming of microbes and planktons	May induce spread of disease and reduce water quality
Phosphate	Eutrophication	Excessive growth of algae prevent light and oxygen from reaching into water leading to untimely death of fishes and other aquatic lives
Salt	Soil salinity	Can render soil unproductive, consequently unarable
Pathogenic microbes	Disease infestation	This is capable of causing food and water contamination, can enter

		body directly through cavities to cause disease
Carbon dioxide and Methane	Global warming	These are greenhouse gases that puncture protective ozone layer to cause climate change. Excessive flooding, heat and drought are manifestations of global warming
Heavy metals	Soil and surface water contamination	Successive accumulation of heavy metals in bodies of human beings and animals through food chain adversely affect its health status

4.0 CONCLUSION

There are two sources of animal waste. Range and pasture animal production system disperse animal waste faster than the confined system. Animal waste has significant value improving crop production and soil sustainability. Over application of animal waste is nuisance and harmful to soil and water ecosystem and human life.

5.0 SUMMARY

In this unit, we have learned that:

1. Animal waste products are such material excreted out of the body of live animal, such as urine, faeces, fur, hair etc.
2. There are two sources of animal waste: the primary and the secondary
3. Animal waste can be dispersed from one place to another through: range and pasture practice, confined practice of animal production, releases from processing industries and or by transportation by man
4. Animal waste can be used for sustained integrated soil fertility management and crop productivity
5. Undue application of manure can cause environmental pollution and disease infestation.

6.0 TUTOR – MARKED ASSIGNMENT

1. Briefly, and convincingly, discuss on the two sources of animal wastes
2. Animal waste is a great soil amendment. Justify?
3. Comprehensively explain why animal waste should not be over-applied
4. In your own view, should ISFM be adopted?

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UNIT 2 SEWAGE MATERIAL

CONTENTS

1.0 INTRODUCTION

Sewage rarely often called wastewater is a suspension of water and solid waste which originates from domestic, municipal and industrial wastes. It is characterized by high toxic substances, and pathologic contaminants. In olden days and nowadays, sewages are directly disposed into water bodies such as rivers and lake and on land without any treatment. Today, the method for sewage and sludge disposal has shift paradigm, which is to direct sewage wastes to wastewater treatment plant. The concern and restriction of the unregulated disposal of sewages wastes is basically due to its high potential to contaminate waters sources and atmosphere, which pose serious health risk to man and animals.

2.0 OBJECTIVES

At the end of this unit, we shall be able to

1. Define the term sewage
2. Describe various processes involve in sewage treatment
3. Know the health risk potential associated with untreated sewage disposal
4. Agricultural benefit of treated sewage

3.0 MAIN CONTENT

3.1 Principles of sewage conversion/treatment

The increase in uncontrolled disposal of sewage materials on resource-based environmental component (soil and water), especially in the phase of rapid population growth has been a daunting challenge against safer ecosystem. This is more severe in urban and semi-urban settlements where immigration adds to the population density. To this end, the idea of converting sewage wastes in to less toxic or usable material became the focus. Consequently a standard was set by environment regulatory bodies; that disposal of sewages into water or on land is permitted only if it does not:

- cause excessive ground water pollution
- pose a direct public health hazard
- accumulate hazardous chemicals in the soil or water that can get into the food chain
- cause accumulation of environmental pollutant that generate unpleasant smell and
- bring about aesthetic loses

To comply effectively with the above stated standard, industries use various technology to remove toxic and offending substances before disposal

3.2 Sewage treatment

For centuries, history has it that, people have recycled human waste by applying in soil to exploit the nutrients and beneficial organic material it contains. However, because of spread of disease, loss of aesthetic value, cultural norms and values, the practice was not well adopted. A pragmatic method of sewage treatment was then developed. This involves the following phases:

Pre-treatment

In this stage, only materials that can be easily collected from the raw sewage are removed. This includes all large material capable of damaging or clogging the pump and sewage line of primary treatment. They include trash, tree climbs, leaves, large cans, packets. This is achieved through the use of bar screen to retain all the large objects. Bar screen of varying sizes is sometimes used to optimize the efficiency of sieving.

Grit removal

This is performed by a grit chamber. The velocity of incoming sewage is lowered to allow for adequate settlement of sand, gravels, crumbs and other solid material. It is aimed at, essentially, reducing the formation of heavy accumulation of sand in aeration tanks and other machine, and also to protect moving machines and lines from undue abrasion. The opening of the grit system retains all solid greater than 0.2 mm.

Primary treatment

Consist of temporarily holding basin, where heavy solids can settle at the bottom, while oil, grease and lighter solids float on the surface. The settled and floating materials are then separated by decantation. The liquid is discharged to the secondary treatment unit.

Secondary treatment

Here both dissolved and suspended biological matter is removed. This is typically performed by indigenous/local micro-organism. The microorganism is thereafter removed from treated water prior to it for tertiary treatment. In stage, biological content derived from human waste, food waste, soaps and detergents are highly degraded. The bacteria and protozoa introduced consume biodegradable soluble organic contaminant e.g. sugar, fat, carbon molecules as energy sources and substrate and bind much of the insoluble ones to coagulate.

Tertiary treatment

This is where the water is disinfected chemically by the chlorination ultraviolent light, sodium hypochlorite, or physically by microfiltration. The disinfection is otherwise called “effluent polishing”.

Fat and oil removal

In some large plants, fat and greases are removed by passing the sewage through a small tank where skimmers collect the fat floating on the surface. Air blowers in the base of the tank may also be used to help recover the fat as froth.

3.3 Sewage as a fertiliser source

Application of sewage sludge could improve soil productivity if properly treated. Adoption of such a green technology comprised of addition of disinfectant, antiseptics and incorporation into bio-fertilizers. It can be used as an approach for reclamation of degraded and marginal lands without causing any environmental damage. However, bio-fertilizers produced through the use of use should be fortified with mineral N and other deficient nutrient for optimum performance. It can be prepared in similar way to compost but require high extent of microbial decay before the manures are added to the soil. Sewage effluent can be applied effectively using sprinkler that have large nozzle openings e.g. sprinkler.

The advantages of this green technology include: reduction of volume in the waste material; stabilization of the waste generated; destruction of pathogens in the waste material and production of biogas for energy use. However, emission of greenhouse gases, notably methane, and Carbon dioxide associated with this technology is main drawback. Sewage treatment can consider alternative sources of synthetic fertilizer. Sewage resources are always readily available and cheap. Let's convert waste to wealth!

- Nitrogen can be generated by Nitrification process
- Phosphorus can be generated by the use of polyphosphate accumulating organisms or chemically by the use of precipitation with salt of iron (Fe) like ferric chloride or aluminium.
-

3.4 Hazardous effect of disposing untreated sewage

Sewage Contain live pathogens (viruses and bacteria, fungi), eggs of intestinal worms, soluble salt, heavy metal e.g. zinc, cadmium, nickel, lead, copper. These can cause heavy out-break of diseases like diarrhoea, cholera, rashes, stomach disorder, kidney failure, cardiac arrest, intestinal parasite *et cetera*.

4.0 CONCLUSION

Sewage heavily contains toxic and pathogenic substance. It can be converted to less toxic before disposal to receiving environment or even be used for agricultural production.

5.0 SUMMARY

In this unit, we have learned that:

1. Sewages are mixture of water and solid waste generated from human bodies, industries and municipal area.
2. Most of its content chemicals and biological organisms could cause heath - hazard and environmental pollution. Treatment of sewage entails a number of process and stage – pretreatment stage, primary, secondary and tertiary stages.
3. Conversion of sewage into less toxic and usable form is referred to as sewage treatment

6.0 TUTOR–MARKED ASSIGNMENT

1. In summary, describe the various sources of sewage
2. Explain the rationale behind sewage treatment
3. List and explain all the processes involved in sewage treatment
4. How does treated sewage relate to agriculture?
5. Mention five health risks associated with improper disposal of an untreated sewage
6. As a supervisor, what possible considerations do you have to take to allow for disposal of sewage into water bodies?

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UNIT 3 PETROLEUM HYDROCARBONS IN SOILS

CONTENTS

1.0 INTRODUCTION

Petroleum hydrocarbons (PHCs) are one of the notable organic contaminants found in the organic wastes. The PHCs is believed to originate from long-term decayed organisms (plant and animal) under a subjection of high temperature and got transformed through complex chemical re-arrangement. They are complex substances that are polycyclic, highly lipophilic. The non-viscous fraction of PHCs is easily absorbed by plants. The unit chemical structure is made up of hydrogen, and carbon .Minute impurities of oxygen, sulphur, and nitrogen also exist in its structure. The two basic elements (C and H) are arranged in infinite number of structures. The carbon atom may be bonded to up to four different carbon atoms called carbon chain/backbone. On the other hand, the hydrogen atoms are bonded to only one carbon atom. Petroleum hydrocarbons are made up of many different compounds which have specific properties. Because of the vast nature the PHCs only general properties of a group is used to predict the properties of a given compound. Chemical Products that occur in PHCs include hexane, benzene, toluene, xylenes, naphthalene, fluorine, gasoline.

2.0 OBJECTIVES

At the end of this unit, students should be able to:

1. Describe petroleum hydrocarbon, its basic products and chemical composition
2. Itemize some important properties of PHCs
3. Explain the effect of PHCs on soil ecosystem
4. Discuss the concept of bio-remediation

3.0 CONTENTS

3.1 Properties of petroleum hydrocarbons

- Volatility: refers to its potential to evaporate when boiled at normal ambient temperature and pressure
- Flammability : How readily a hydrocarbon starts to burn
- Octane number: an indication of their resistance against detonation/ knocking
- Presence of impurities: PHCs contain a number of impurities like gums, metals, microbes, sediment load, sulphur and water of condensation

3.2 Effect of petroleum hydrocarbons on soil

Petroleum Hydrocarbons (PHCs) affect food security by rendering the soil uncultivable/ unarable and groundwater unsuitable for irrigation purpose. The PHCs cause decrease in agricultural productivity of the soil in various ways. They primarily, deplete oxygen content

in the soil which affect microbial activities and root respiration, causes soil caking that reduces soil consistence, create surface coating that hinders water and nutrient infiltration and enhanced fixation of available nutrient, thus rendering the soil infertile. For instance, in Nigeria, soil affected with PHC records drastic reduction in total nitrogen, available phosphorus and also showed high values of C: N and C:P ratio, electrical conductivity, and pH. Health risk is a secondary effect in farming system which occurs through direct contact with the PHCs-contaminated - soil which reduce human labour productivity and availability. They as well contaminate water reservoirs underlying the soil.

The toxicity of PHCs to microorganisms, plants and meso animals (earth worms, millipedes) inhabiting the soil does serious harm to its suitability to support crop growth and development. The toxic effects of hydrocarbons on terrestrial higher plants and soil microbes have been ascribed to their ability to dissolve the lipid in the cytoplasmic membrane, thus allowing cell contents to drain.

3.2.1 Sources of PHCs

- **On-site sources**

Oil/gas industry also known as petroleum industry releases PHCs in the sites of exploration, extraction, refining and storage

- **Off- site**

The PHCs get released onto the soil environment in the course of transportation and marketing of petroleum products. This may be through breaking of installation pipes, frequent accidents by the trucks loaded with PHCs and fire outbreaks that affects filling station.

3.3. The concept of bioremediation

Bio-remediation generally entails employing different ways to treat contamination through microbes and flora. Bio-remediation can occur naturally or can be encourage with addition of microbes and fertilizers. It has been known over the years that certain microorganisms are able to degrade petroleum hydrocarbons and use them as a sole source of carbon for energy and growth. Many methods were evolved for remediation of contaminated soil, which include physical (e.g. excavation, earth-filling, washing), chemical (*e.g.*, soil vapour extraction, sodification/stabilisation) and biological methods. But biological methods are economically cheaper, safer and more efficient then chemical and physical ones. However, the rate at which biological remediation is achieved is naturally slower compare to physical and chemical methods. As such, large numbers of methods have been developed to increase the degradation rate of petroleum products in soil by microbes. In comparison to other biological methods, bioremediation through microorganism is more efficient, but the low solubility and adsorption of high molecular weight hydrocarbons limits their availability to microorganisms.

The soil microbes use the hydrocarbon present in the petroleum as a source of energy and carbon, thereby breaking it down through enzymatic release. The low solubility and adsorption of high molecular weight hydrocarbons limit their availability to microorganisms. To overcome this limitation biosurfactants are added to fasten the solubility and removal of these contaminants, thus, enhancing PHCs biodegradations rates. To ensure quick and efficient bioremediation, the following may be recommended:

i. New species that have the genetic potential for the bioremediation could be introduced
ii. Nutrients required by the microbes such as nitrogen, phosphorus should be supplemented to facilitate their activities and increase their population (Table 6).

iii. Addition of facilitator substances that will enhance bio-availability and reduce degradability of the PHCs e.g. should be considered (Table 7).

Table 6: Composition of a microbial cell

Element	Percentage	Element	Percentage
Carbon	50	Sodium	1
Nitrogen	14	Calcium	0.5
Oxygen	20	Magnesium	0.5
Hydrogen	8	Chloride	0.5
Phosphorous	3	Iron	0.2
Sulphur	1	All others	0.3
Potassium		1	

Source: Stainer *et al.* (1986). In: Vidali (2001).

Table 7: Environmental requirement affecting bio-degradation

Parameters	Condition required for microbial activity	Optimum value for an oil degradation
Soil moisture	25–28 % of water holding capacity	30–90 %
Soil pH	5.5–8.8	6.5–8.0
Oxygen content	Aerobic, minimum air-filled pore space of 10 %	10–40 %
Nutrient content	N and p for microbial growth	C: N: P = 100: 10: 1
Temperature (°C)	15 – 45	20 – 30
Contaminants	Not too toxic	Hydrocarbon 5–10% of dry weight of soil
Heavy metals	Total content 2000 ppm	700 ppm
Type of soil	Low clay or silt content	

Source: Vidali (2001)

4.0 CONCLUSION

Petroleum hydrocarbons (PHCs) are soil contaminants and therefore reduce its productivity drastically. There are two sources through which get into soil – On- site and off-site. Contaminated soils can be remediated.

5.0 SUMMARY

In this unit, we have learned that:

1. Petroleum hydrocarbons originated from decayed bodies of prehistoric organism
2. PHCs are composed mainly of carbon and hydrogen elements, having complex chemical arrangement
3. Soil contaminated with PHCs are not productive but can be remediated through various mean including bio-remediation
4. Dissolution of the cytoplasmic-membrane lipid is one way through which PHCs destroys the bodies of plant and microorganisms

6.0 TUTOR- MARKED ASSIGNMENT

1. Using five short sentences explain what you understand by petroleum hydrocarbons.
2. From the context of soil PHCs interaction, how did PHCs negates food security?
3. Write short-note on bioremediation.
4. Assuming a bacterium Bti, is to be used for bio-remediation and it requires nitrogen for its growth and development, which fertiliser type will you recommend for application?

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UNIT 4 DETERGENTS IN SOILS

CONTENTS

1.0 INTRODUCTION

Soap ineffectiveness in removing dirt and oil and formation of scum in hard water resulted in development of detergents. It was first developed around 1950s. Detergent or surfactant (a surface active agent) is any non-soap cleaning agent especially a synthetic material with cleaning properties in dilute solution. They are similar to soap but are more soluble in hard water. They are usually used for domestic purposes such as laundry and dish washings, biological and medical purposes as reagent for isolation and purification and cleansing. They also used as fuel additives to prevent fouling (accumulation of unwanted materials on solid surface that reduce machine efficiency of machines) . Surfactants are added to enhance mobility of pesticides particularly diazinon, atrazine, metolachlor and acephate in soil which play a decisive role in the strength and selectivity of the pesticide effect. They as well successfully employed for the enhancement of the efficacy of the active ingredient in pharmaceutical formulations. Detergents are produced either in solid form such as powder or liquid form as concentrated solution. They act basically because of their amphiphilic property - partly hydrophilic (polar) and partly hydrophobic (non-polar). They have sodium salt of long chain alkyl benzene sulphonic acids or sodium salt of long chain alkyl hydrogen sulphate.

2.0 OBJECTIVES

At the end of this unit, students should be able to:

1. Know what is meant by detergents
2. Classify detergents and its mode of action
3. Explain how soil and detergent interact
4. State various fate of detergents in the soil
5.

3.0 MAIN CONTENT

3.1 Classes of Detergents

Detergents are classified into anionic detergents e.g. Alkylbenzenesulfonate, cationic detergents and non-ionic and witter detergent e.g. Tween, Triton.

3.1.1 Mode of action

Detergents basically reduce surface tension of water molecules to be able to thoroughly wet, soak and seep into clothes and fibres. They also have the ability to emulsify oil and grease

3.1.2 Soils-Detergent Interaction

Some chemical ingredients of detergents are persistent organic pollutant that can remain in soil for years not degraded, implying their tendency to have residual effect. Chlorine, sodium and boron found in detergents have harmful effect on soil. Sodium compounds damage soil structure by causing dispersion and precipitation of soil particles. It is generally accepted that soil structure and texture are the most important properties governing soil transport. At higher concentration of surfactant the soils hydraulic conductivity is significantly reduced. The effect depends on its concentration and the clay content. Rise in alkalinity of soil associated with chlorine may limit availability of critical soil nutrients and make the soil environment less conducive for soil beneficial soil fauna. In soil- detergent interaction capillary forces phenomenon of dried soils has a considerable impact on the mobility of surfactants, hence exposing the soil to more damage. Because of complexities of the soil-detergent interaction, models for prediction detergent sorption were not successful. High variability exists between various detergent types in terms of adsorption capacity and biodegradability of surfactants in soil. For instance, Ethoxylated anionic surfactants showed lower adsorption capacity than nonethoxylated anionic and nonionic surfactants; however, these surfactants were easily biodegradable. Soil temperature also exerts a marked influence on the behaviour of anionic surfactants in soil.

3.2 Fates of Detergents in the Soil

Various compounds of detergent are subject to the following fates

1. Absorption by living plants
2. Immobilization and complexation
3. Percolation to ground water
4. Evaporation to the atmosphere

4.0 CONCLUSION

Detergent has the ability to clean grease and oil from surfaces. It also generates forms in hard water (water with high salt). Soil interacts with detergent in a very complex manner. Detergents in soil are predisposed to various fates.

5.0 SUMMARY

In this unit, we have learned that:

1. Detergent are synthetic product containing a mixture of surfactant that has the ability to remove grease and oil and generate form in hard water
2. Reduction of surface tension of water and emulsification are the basic mode of action of detergents
3. Some chemical elements in detergents notably sodium and chlorine affect soil structural stability and acidity respectively.
4. Compounds of detergents in soil could be, absorbed, immobilized, percolated or evaporated

6.0 TUTOR –MARKED ASSIGNMENT

1. In a sentence state what necessitates the development of detergents
2. Itemize the categories of detergents
3. List three uses of detergents
4. Soil-Detergent interaction. Elaborate

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UNIT 5 PESTICIDES IN SOILS

CONTENTS

1.0 INTRODUCTION

Any substance (liquid, gas or solid) that is used to kill or suppress pest is known as a pesticide. Pest refers to any living organism that is capable of causing harm, inflicting or inciting disease to another organism. However, the name "pesticide" is most often used to refer to synthetic chemical used to kill pest. Since, 19th century, the use of chemicals such as bitumen fumes, vinegar, tobacco water and copper sulphate has been employed to kill pest. In the early 19th proliferation of synthetic herbicide such DDT (dichlorodiphenyltrichloroethane), organo-phosphate, phenoxyacetic came with full force. Many pesticides are applied directly on the soil surface, while some on plant foliage, which also return to the soil. The process by which chemical mixtures are formed to effectively control a pest is termed pesticide formulation.

2.0 OBJECTIVES

At the end of this unit, students should be able to:

1. Describe what pest and pesticides are
2. Distinguish the role of the two component of pesticide
3. To categorize different kind of pesticide
4. Elaborately explain various fates of pesticides in the soil

3.0 MAIN CONTENT

3.1 Composition of pesticide

Pesticide is a mixture of active and inert ingredient (carrier). The former is any substance that kill, repel or prevent a pest e.g. pyrethrins, octyl bicyloheptane, while the latter is a solid or liquid substance which does not possesses the pesticidal properties but aid in the application of the active ingredient and most often help to make the pesticide stick on the applied surface.

3.2 Categories of pesticide according to target pest

Herbicides - to suppress weed plants
Insecticides – to suppress insects

Fungicide – to suppress fungi
Rodenticide – to control rodents
Bactericides – to kill bacteria
Larvicides – to kill larvae
Molluscicide – to control snails, and molluscs

3.2.1 Category of pesticides based origin

Chemical e.g. endosulfan, endrin and biological pesticide (botanicals) e.g. rotenoid, nicotinoid, neem oil, canola

3.2.2 Category based on formulation

- Organo-chlorines e.g. DDT (dichlorodiphenyltrichloroethane), HCHs (hexachlorocyclohexane), endrin, aldrin etc. They are highly persistent and has bio-accumulation potential
- Organo-phosphate e.g. Parathion, Malathion, Methyl parathion
- Carbamate e.g. Cabofuran, Propoxur, Methomyl

3.2.3 Category based on mode of action

- Systemic: The pesticide is soluble and readily absorbable by pest and moved within the body system of the target pest. It operate mainly by tempering with the metabolic activities and various body systems of the pest e.g. acephate, dinotefuran
- Non systemic: This category act only externally. They are directly applied onto foliage, buds, roots, fruits. The primary target of non-systemic pesticide are chewing and flying insect. They act when in contact with the target pest e.g. bifenthrine, neonicotinoid

3.2.4: Category based on selectivity

- Non- selective Pesticide: Otherwise known as broad-spectrum. This kind of pesticide kills wide species of plants and insects. It's usually applied as post emergence pesticides to clear the entire field, after all the expected weeds might have emerged. Later, crop of interest is sown.
- Selective Pesticide: Also referred to as narrow spectrum, because they act on specific target weed and insect. They are usually applied to control pest during the growth stage of crop plant.

3.3 Fates of pesticides in the soil

- **Absorption by plant**

Some molecules of pesticide are readily absorbed by plant and therefore prevent its movement into groundwater. Maize and Amaranths are typical examples of phyto-accumulator capable of absorbing large amount of pollutant.

- **Physical Protection**

Some molecules may be physical caged by soil colloids in the process called immobilization. Texture, pH, water status, organic matter and chemical properties of soils are the major controlling factor of physical binding ability of soils. Some soil chemical compound may also form complex chemical product with pesticide molecule in the process called complexation. This prevents their leaching and subsequently makes them susceptible to microbial degradation.

- **Washing**

Pesticides added on to soil surface could be carried away by moving water. Highly soluble pesticides have the greater tendency of being washed away by surface overflow. High

velocity water added on to the soil either through irrigation or precipitation tends to substantially washed away applied pesticide along furrows and sloping terrain by preferential flow

- **Percolation to ground water**

This is more pronounced in soil with high infiltrability. Soil with predominance of macropores could facilitate downward movement of applied pesticide into ground water to cause contamination when the soil is wetted.

4.0 CONCLUSION

Pesticides are used to control pest. Active and inert ingredients are the main component of a pesticide. Pesticides are categorized on several bases. Four fates of pesticides in the soil are recognized – absorption, immobilization, washing, and percolation to ground water

5.0 SUMMARY

In this unit, we have learned that

1. Pest is any organism that is capable of causing harm to other organism called the host
2. Pesticides refers to all substances applied in order to control pest
3. Pesticides undergo various fates in the soil

6.0 TUTOR-MARKED ASSIGNMENT

1. What are Pests?
2. Pesticides and its classification based on selectivity, highlight
3. Give concise overview on pesticide
4. State seven categories of pesticides based on target pest
5. As a farmer, you observed an emergence of wide variety of weed species on your farm prior to sowing, which pesticide will you recommend for total control? State clear reason (s) for your choice.
6. As an agricultural field worker, a farmer asked you, whether to irrigate his farmer heavily immediately after he applied a pesticide, what may be your response, and why?
7. Give two examples each of Organo-phosphate, Carbamate and organo-chlorine pesticides

7.0 REFERENCES/ FURTHER READINGS

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