**UNIT- II**

Microbiology and biochemistry of root-soil interface; phyllosphere; soil enzymes, origin, activities and importance; soil characteristics influencing growth and activity of microflora.

**Microbiology and biochemistry of root-soil interface**

**The Rhizosphere Defined**

Lorenz Hiltner (1904) described the rhizosphere as the area around a plant root that is inhabited by a unique population of microorganisms influenced, he postulated, by the chemicals released from plant roots. In the years since, the rhizosphere definition has been refined to include three zones which are defined based on their relative proximity to, and thus influence from, the root (Figure 1). The endomycorhizosphere includes portions of the **cortex** and **endodermis** in which microbes and cations can occupy the “free space” between cells (apoplastic space). The **rhizoplane** is the medial zone directly adjacent to the root including the root epidermis and mucilage. The outermost zone is the **ectorhizosphere** which extends from the rhizoplane out into the bulk soil. As might be expected because of the inherent complexity and diversity of plant root systems (Figure 2), the rhizosphere is not a region of definable size or shape, but instead, consists of a gradient in chemical, biological and physical properties which change both radially and longitudinally along the root.

## http://www.nature.com/nrmicro/journal/v11/n11/images/nrmicro3119-i1.jpgApoplastic and symplastic compartments in plants: Plant tissue is divided into the symplastic (also known as cytoplasmic) compartment on the inner side of the plasma membrane and the apoplastic (also known as extracellular or cell wall) compartment on the outer side. Plasmodesmata, which are microscopic channels that enable the transport of small molecules and some proteins between plant cells, connect the cytoplasms of most cells in plant tissue to form a symplastic continuum throughout the plant (see the figure; not drawn to scale). The size exclusion limit of proteins that can pass through plasmodesmata varies depending on plant cell type. Guard cells lack plasmodesmata altogether. Plant cells typically contain a large vacuole separated from the cytoplasm by a vacuolar membrane, known as the tonoplast. Incubation of plant tissue in hypertonic solutions (such as molar sucrose solution) results in plasmolysis[90](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref90),[123](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref123), during which water is drawn by osmosis from the cytoplasm and vacuole into the apoplast, and the plasma membrane and enclosed protoplasm shrink away from the cell wall. The accumulation of extracellular defence responses seems to require plasma membrane–cell wall connections[90](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref90). In animal cells, the extracellular matrix–plasma membrane–cytoskeleton continuum is maintained by receptors that recognize RGD-containing matrix proteins, and RGD-proteins also seem to be important in plants. The plant plasma membrane–cell wall continuum is visible as Hechtian strands, which are thin connections between the protoplast and cell wall, after plasmolysis. Gentle, stepwise plasmolysis, which maintains Hechtian strands and is reversible[90](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref90),[123](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref123),[124](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref124), can be achieved by the stepwise addition of hypertonic solutions to tissue on a microscope slide[54](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref54),[90](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref90),[123](http://www.nature.com/nrmicro/journal/v11/n11/full/nrmicro3119.html#ref123). Plasmolysis shows that the plasma membrane remains intact as the pathogen grows inside host cells and defines the relationship of hyphae to the apoplast. To assay cytoplasmic translocation, plasmolysis separates putative cytoplasmic fluorescence from autofluorescence in cell walls and enlarges the apoplastic space for better differentiation of apoplastic from cytoplasmic localization.



Figure 1. Schematic of a root section showing the structure of the rhizosphere.

The practical definition of rhizosphere soil is that soil which adheres to or is influenced by the root but which can be removed from the root by gentle shaking in sterile water. Rhizoplane soil is that which is obtained when the roots are transferred to a fresh sterile solution and shaken vigorously. A control or bulk soil is soil which does not adhere to the plant root and is not influenced by the root. Although the rhizosphere, obviously extends into the soil for some distance, the total volume of rhizosphere soil is difficult to assess. The rhizosphere volume can be altered by plant species, soil type, soil moisture, portion of the root being evaluated, and the method used to determine the rhizosphere volume. To quantitate the rhizosphere effect, an R/S ratio has been used. The R/S ratio is determined by dividing the number of microorganisms (or the rate of a biochemical process) per gram of rhizosphere soil by the number of microorganisms in a g of the control (bulk) soil.

The **rhizosphere** is the narrow region of [soil](https://en.wikipedia.org/wiki/Soil) that is directly influenced by [root](https://en.wikipedia.org/wiki/Root) secretions and associated soil-[microorganisms](https://en.wikipedia.org/wiki/Microorganism). Soil which is not part of the rhizosphere is known as [bulk soil](https://en.wikipedia.org/wiki/Bulk_soil). The rhizosphere contains many [bacteria](https://en.wikipedia.org/wiki/Bacteria) that feed on sloughed-off plant cells, termed *rhizodeposition*, and the proteins and sugars released by roots. Protozoa and nrematodes that graze on bacteria are also more abundant in the rhizosphere. Thus, much of the nutrient cycling and disease suppression needed by plants occurs immediately adjacent to roots.

Factors responsible for development of the Soil-Plant Root Rhizosphere

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Release of soluble organic compounds by plant roots

Sloughed off root cell debris and dying root hairs

Plant root cell lysis

Higher concentration of carbon dioxide

Lower concentration of oxygen

Lower concentration of nutrient ions

Partial desiccation of soil due to absorption of water by roots

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**Interactions**

Roots are important not only for water and nutrient supply of the plant, but also to release a wide range of carbon compounds of low molecular weight, such as sugars, amino, and organic acids. These can amount to between 10% and 20% of total net fixed carbon (total photosynthate ranges from 30% for cereals to nearly 60% for some trees) but vary based on species, nitrogen availability, and plant age. In addition, most land plants form symbioses with soil fungi, which in addition cause a considerable drain of photoassimilates. Direct (plant exudates) or indirect (via symbiotic fungi) rhizodeposition of carbon forms the basis for an environment rich in diversified microbiological populations.

The rhizosphere is now defined as a narrow zone of soil, which is influenced by living roots. It forms a boundary layer between the root and the bulk soil. Here large fluxes of solutes and water, as well as compounds contained in the gas phase, exist. Consequently, physical soil properties can vary considerably. Depending on the demands of the plant, changes in the soil water potential can be high during the day/night cycle. In comparison to bulk soil, the soil water potential can become strongly negative during the day at high transpiration rates and less negative at night because of vertical redistribution by the root system (hydraulic lift). Special conditions also exist with regard to O2 and pH. Following high rates of respiration by both roots and microorganisms, O2 tension can be very low especially in wet soil where water limits diffusion rates. Uptake of solutes is often accompanied by the release of protons and organic acids, which affects the pH at the root surface.

Microorganisms of the rhizosphere establish a functional diversity that includes the decomposition of organic matter, nitrogen fixation, conversion of inorganic forms of nitrogen, solubilization of phosphate, transformation of sulfur and iron, production of siderophores (iron-binding compounds), release of plant (phyto)hormones, as well as of compounds, which are used for biotic control.

It is obvious that bacteria are an important part of the microorganisms inhabiting this ecological niche. In comparison to bulk soil, the abundance of rhizosphere bacteria is several magnitudes higher (1010–1012 microbes per gram soil versus <108 in bulk soil), but still about 100 times lower than under culture conditions. Bacteria can solubilize nutrients from the mineral soil layer, but will also sequester them. Consumption of bacteria by soil protozoa and nematodes will then liberate nutrients, which in due course will become available for plants.

Fungi form another important part of the rhizosphere. Most terrestrial plants develop symbiotic structures (mycorrhiza) with soil-borne fungi, creating another sphere, the mycorrhizosphere. In these interactions, the fungal partner provides the plant with improved access to water and soil nutrients because of more or less complex hyphal structures, which emanate from the root surface and extend far into the soil. The plant, in return, supplies carbohydrates for fungal growth and maintenance. Because of leakage and the turnover of mycorrhizal structures, these are another source for solutes released into the soil where they can be accessed by other microorganisms.

In the following interactions of bacteria, fungi and plants, and, finally those of plants with each other are addressed. With regard to soil bacteria, a wide range of bacterial activities exist such as the “good” ones (plant growth promotion, plant disease suppression, nitrogen fixation) and the “bad” ones (plant pathogens), as well as bioactive compounds of bacterial secondary metabolism, which cause the respective effects. Fungi form another focus, here especially the symbiotic and plant pathogenic fungi. Finally, direct (parasitic plants, plant competition) and indirect (by the help of fungi) interactions of plants themselves are described. Three partite systems (host plant + two microorganisms (e.g., pathogen + antagonist)) become already very difficult to handle, and the step to field studies is enormous.

**Changes in soil properties in the rhizosphere**

The soil becomes more and more influenced by a root the closer one gets to the root surface. The interface between root and soil (rhizosphere) is a complex zone.

*The main soil properties that are likely to be influenced by root growth and activity*

**Bulk density** - if roots have to enlarge pores in penetrating the soil, the surrounding soil will be compacted by compression and re-orientation of particles.

*Compression of soil by plant roots*

|  |  |  |
| --- | --- | --- |
| Soil | Initial density (g cm-3) | Final density (g cm-3) |
| Fine sand | 1.401.25 | 1.501.60 |
| Sandy loam | 1.30 | 1.60 |
| Loam | 1.50 | 1.53 |
| Clay | 1.21 | 1.30 |

**Moisture content** - in most agricultural soils during the growing season the water content around a root will be less than that in the bulk soil because of the plant demand due to transpiration. However, water can also move out of the root, and some 'pumping' of water from deep in the subsoil can result in localized re-wetting of dry soil around the roots of desert plants.

**Nutrient concentration** - for immobile nutrients there will tend to be a concentration gradient that increases with distance from the root. This gradient will tend to be much less pronounced for mobile nutrients, and the concentration may decrease from the root surface if the nutrient moves to the root faster by mass flow than it can be absorbed.

**pH** - net H+ extrusion into the apoplasm will lead to pH decreasing in the rhizosphere. In contrast, the net release of HCO3- into the apoplasm or net uptake of H+will lead to an increase in pH.

The effective change in pH of the rhizosphere will depend on the *anion:cation uptake ratio*.

anion:cation > 1 pH increases
anion:cation < 1 pH decreases
pH at the root surface can change as much as 3.5 units.

The pH change is often determined by the form of N supplied: NO3-N or NH4+-N.

N is the only nutrient that is absorbed in both the anion and cation forms (eg K+ is always absorbed as a cation).

NO3 is the most common form of mineral N in the soil.

Norg => NH4+

2NH4+ + 3O2 =>2NO2+ 2H2O + 4H+

(the release of protons causes the pH to decline)

2NO2+ O2 => 2NO3

**pH changes**

The change in pH will affect phosphorus absorption particularly but other ions as well eg. Mn, Fe, Al, Zn.

* *phosphorus availability and pH effects in the rhizosphere of corn*

|  |  |  |
| --- | --- | --- |
| Fertilizer regime | pH of rhizosphere | P in shoot g/mg |
| MCP (mono-calcium phosphate) | 7.3 | 0.31 |
| MCP + K2SO4 | 6.9 | 0.61 |
| MCP + (NH4)2SO4 | 6.7 | 1.04 |

Acidification increases P solubility and the proportion absorbed.

 pKa = 7.2              pKa = 12.3
H2PO4- <=> HPO42 - + H+ <=> PO43 - + H+

The effect of a reduction in pH of the rhizosphere on the balance between H2PO4- and HPO42 - is of importance for neutral or alkaline soils. But if the pH is less than 6, little of the P will be in the HPO42 - form, so further reduction will have negligible effect on the amount of each form present.

* *implications for fertilizer recommendations (*In neutral or alkaline soils)

If N can be maintained in the NH4+-N form, this will enhance the uptake of P.
So for banded fertilizers, including NH4+-N will increase P availability.

Increasing anion uptake will increase pH, and this can lower P availability.

*Effect of enhanced anion uptake on P uptake in Brassica napus* (of a P deficient soil)

|  |  |  |
| --- | --- | --- |
| Anion:cation uptake ratio | pH of rhizosphere | P in solution g/mg |
| >1 (more anions) | 6.5 | 0.82 |
| <1 (more cations) | 5.3 | 1.40 |

  ...

|  |  |  |
| --- | --- | --- |
|  | pH of rhizosphere | P in solution g/mg |
| MCP + CaCl2 | 4.3 | 0.6 |
| MCP + (NH4)2SO4 | 4.1 | 1.1 |

 However, in acid soils, where P is adsorbed to Fe or Al oxides, enhanced availability of P can result if the phosphorus can be exchanged at these sites with bicarbonate ions.

Studies using soil infiltrated with agar containing a pH indicator have demonstrated interesting effects.

1. Rhizosphere pH was reduced by chickpea but increased by sorghum when both were growing in the same pot supplied with NO3--N. This is still a function of the imbalance of anion and cation uptake. Cereals tend to take up much more silicon than do dicots, especially legumes. The net result is that with NO3--N as the nitrogen source there will be a greater tendency to raise the pH of the rhizosphere in cereals, but the greater demand for cations in legumes will still tend to result in further acidification.
2. Rhizosphere pH was increased along the main axis and decreased along lateral roots of corn growing in a soil supplied with NO3-N. Localized differences in the anion:cation uptake ratio appear to be important.

**Redox potential** - roots absorb oxygen, thereby generating a concentration gradient away from the root surface. However, in waterlogged soils the internal transport of oxygen can be sufficient that leakage into the rhizosphere is sufficient to maintain an oxygenated zone around the root. In rice, the oxidation zone may extend up to 4 mm from the root surface.

Fig. 1 Production and consumption of N2O and CH4 (components of GHG) in the rhizosphere of wetland plants

**Effects on other soil properties**

The growth of roots affects many soil properties because of the changes in soil water and nutrient contents. A number of these effects in combination can have a significant effect on soil structure and structural stability.



**Organic matter** - the sloughing of cells from the root cap, the secretion of plant mucilages, the release of organic compounds, and the turnover of fine roots will all add to the organic matter in the soil around a root. Mucigel has also been shown to bridge clay particles thereby increasing the cohesion of soil particles and leading to the formation of micro-aggregates.

## Secretions

Plants secrete many compounds through their roots to serve symbiotic functions in the rhizosphere. Strigolactones, secreted and detected by mycorrhizal fungi, stimulate the germination of spores and initiate changes in the mycorhiza that allow it to colonize the root. The parasitic plant, *Striga* also detects the presence of strigolactones and will germinate when it detects them; they will then move into the root, feeding off the nutrients present. Symbiotic Nitrogen-fixing bacteria, such as *Rhizobium* species, detect compounds like flavonoids secreted by the roots of leguminous plants and then produce nod factors which signal to the plant that they are present and will lead to the formation of root nodules. In these nodules bacteria, sustained by nutrients from the plant, convert nitrogen gas to a form that can be used by the plant. Non-symbiotic (or "free-living") nitrogen-fixing bacteria may reside in the rhizosphere just outside the roots of certain plants (including many grasses), and similarly "fix" nitrogen gas in the nutrient-rich plant rhizosphere. Even though these organisms are thought to be only loosely associated with plants they inhabit, they may respond very strongly to the status of the plants. For example, nitrogen-fixing bacteria in the rhizosphere of the rice plant exhibit diurnal cycles that mimic plant behavior, and tend to supply more fixed nitrogen during growth stages when the plant exhibits a high demand for nitrogen.

Although it goes beyond the rhizosphere area, it is to note that some plants secrete allelochemicals from their roots which inhibit the growth of other organisms. For examples, garlic and mustard produce a chemical which is believed to prevent mutualisms forming between the [trees](https://en.wikipedia.org/wiki/Tree) and mycorhiza.

**Root mucilage** - consists 99.9% of water, the water potential of the fully hydrated gel is about -7 kPa. A clay-mucigel combination will hold more water than will the clay alone.

**Release of organic compounds**

A large percentage of the organic carbon in the root rhizosphere is a result of cuticle of the root being lysed by microbes or ruptured by mechanical abrasion. A second important source of carbon in the rhizosphere is the organic material introduced as root exudate or secretion. There is a subtle difference between root exudation and secretion processes. Root exudates are low molecular weight compounds that leak from all cells either into the intracellular spaces and then into the soil or directly through the epidermal cell walls into the soil. The release of these compounds is not metabolically mediated. Secretions are compounds of both low and high molecular weight that are released from the plant root as a result of metabolic processes.

Plants also induce changes in the rhizosphere by releasing organic materials as root exudates. The mucigel present in the rhizosphere contains a mixture of plant and microbial materials. These materials may affect absorption of Fe and Mn and P. The mucigel may also provide some protection to the root against pathogens.

Plants may secrete specific compounds that complex Fe-siderophores. These Fe-complexes may be absorbed intact by the plant increasing the supply of Fe to the plant.

*Effects on release of free compounds*

* Mechanical impedance
* Nutrient deficiency

*Effect on types of free compounds released in the soil*

* Most are organic acids, so they will affect pH in the rhizosphere. They may complex with ions in the soil.
* There is chelation of Fe, Mn and other metals by organic acids. The mucilage of citrus species selectively binds Cu2+.
* Phytosiderophores are specific chelators of Fe3+. They are mainly organic acids such as citrate or phenolics.
* In calcareous soils mucilages are important in iron nutrition since the concentration of iron in solution is very small. Fe deficiency results in the secretion of siderophores.
* In chelating Fe or Al, P can also be released.
* Mn is more mobile in the reduced state, just like Fe, so its availability changes rapidly depending on the redox condition in the soil. Redox potential will be greatly affected by root activity, including the release of exudates, and microbial activity.



* Manganese availability is influenced by the redox potential in the rhizosphere and by root secretions.



* The availability of P, Fe and Mn is affected by a number of different secretions from roots. Their relative importance is indicated in the following table.

**Root secretions and nutrient availability**

|  |  |
| --- | --- |
| Nutrient | Secretion |
|   | OH- / HCO3- | H+ | Ionophore | Chelating agent | Enzyme |
| Fe | - | + | ++ |  |  |
| Mn | - | + |  |  |  |
| P | - | + |  | + | + |

**Microorganism population** - the microbial population in the rhizosphere will usually be much greater than in the bulk soil due to root exudates and cells or root hairs that have been sloughed off by the root.

**Ratio of bacterial colonies in the rhizosphere of various crops to that in the bulk soil**

|  |  |
| --- | --- |
| Crop | Ratio of  colonies in rhizosphere to colonies in bulk soil |
| Maize | 3 |
| Wheat | 6 |
| Barley | 3 |
| Red clover | 24 |
| Oats | 6 |

The following table provides a framework for considering some important changes.

**The likely differences in soil properties between the bulk soil and soil from the rhizosphere**

|  |  |  |
| --- | --- | --- |
| Soil property | Greater (in rhizospheric soil) | Smaller (in bulk soil) |
| Bulk density | Generally yes |  |
| Organic matter | Probably yes |  |
| Moisture content | Can be greater under certain limited circumstances | Generally yes |
| Nutrient concentration | May be greater for cations such as Ca2+ or Mg2+  | Generally less  |
| pH | Depends on root activity, soil and available nutrients | Depends on root activity, soil and available nutrients |
| Redox potential | Depends on soil water content and root response to hypoxia | Generally yes |
| Micro-organism count | Generally yes |  |

One thing is obvious from the table the size and direction of the change in soil properties may vary widely depending on the soil, the crop and their interaction.

**Soil microorganisms**

The mucigel in the rhizosphere provides a favourable mico-environment for microbes. The population density of microbes may be 10 to 200 times greater than that in the bulk soil.

The concentration of soil microorganisms in the rhizosphere can be either beneficial or detrimental to the plant depending on the species that dominate. Microbial activity may increase availability of nutrients by mineralization of organic forms or by increasing the solubility of mineral forms.

Availability of N and P, as well as nutrients like Mn have attracted research into soil microbiology.

*Contribution of grazing protozoa to availability of mineralized-N*

|  |  |
| --- | --- |
| System | Plant uptake (mg N) |
| Wheat + bacteria | 1.61 |
| Wheat + bacteria + protozoa | 2.55 |

* For Mn there are particular bacteria that oxidise the metal ions to form oxides.
* For P there are solubilising bacteria, which have been exploited in China and E. Europe.
* There are also P solubilising fungi eg Penicillium balaji strain (Provide/PB50). This is used on the prairies for wheat and canola.

The most important microbes for P are mycorrhizal fungi that form associations with plant roots.  The majority of plants establish such an association with certain types of soil fungi; this association is known as a **mycorrhiza** ("fungus-root").  Mycorrhizas are generally mutualistic. Carbohydrate is passed from the plant to the fungus, and in return the fungus facilitates increased nutrient uptake, particularly of phosphorus, from the soil to the plant.



Interest in these symbioses has increased dramatically in recent years, because of their potential benefit in agriculture, forestry, and re-vegetation of damaged ecosystems. Some plants cannot become established or grow normally without an appropriate fungal partner. Even when plants can survive without mycorrhizas, those with "fungus roots" grow better on infertile soils and areas needing re-vegetation.

**Phyllosphere**

The term “**phyllosphere**” was first published by Dr. Jakoba Ruinen in 1961. She called the interface between leaves and air as the “phyllosphere”, and said that this was a much neglected milieu, compared to studies of the [rhizosphere](http://en.wikipedia.org/wiki/Rhizosphere).

The **phyllosphere** is a term used in microbiology to refer to the total above-ground portions of plants as habitat for microorganisms. The phyllosphere can be further subdivided into the **caulosphere** (stems), **phylloplane** (leaves), **anthosphere** (flowers), and **carposphere** (fruits). The leaf surface has been labelled the **’phylloplane’** and the zone on leaves inhabited by microorganisms as ‘**phyllosphere’**. The below-ground microbial habitats (i.e. the thin-volume of soil surrounding root or subterranean stem surfaces) are referred to as the **rhizosphere** and **laimosphere**. Most plants host diverse communities of microorganisms including bacteria, fungi, archaea, and protists. Some are beneficial to the plant, others function as plant pathogens and may damage the host plant or even kill it. However, the majority of microbial colonists on any given plant have no detectable effect on plant growth or function.

Research into the characteristics of microbial life in the phyllosphere is of great commercial importance to the agricultural industry for two reasons. First, understanding the survival of plant disease-causing bacteria and fungi is vital for developing new ways to control their spread. Second, there has been a recent rise in the number of food poisoning cases associated with fruit and vegetables contaminated with bacteria, such as *Salmonella* and *E. coli*. This is particularly true of fresh fruits and salads which are not cooked prior to consumption. Preventing these outbreaks by developing better decontamination strategies is important to protect public health.

The bacteria that live on the leaf surface are so numerous that they not only likely affect the plant on which they dwell but, collectively – on a planetary scale – they are so numerous as to significantly affect the global carbon and nitrogen cycles on Earth.

Plant parts, especially leaves are exposed to dust and air currents resulting in the establishment of a typical flora on their surface aided by the cuticle, waxes and appendages which help in the anchorage of microorganisms.  These microorganisms may die, survive or proliferate on leaves depending on the extent of influence of the materials in leaf diffusates or exudates. Leaf diffusates or leachates have been analysed for their chemical constituents. The principal nutritive factors are amino acids, glucose, fructose and sucrose.

Several different epiphytic-fitness traits have been proposed to allow bacteria to colonize the phyllosphere. Among these are metabolic adaptations that enable bacteria to take up and utilize various carbon compounds, including multicarbon compounds such as sugars and amino acids (which can be utilized by various phyllosphere bacteria), and also more specific carbon sources such as methanol. The production of plant hormones, including indole-2-acetic acid (IAA), has been established as an epiphytic factor that might increase nutrient availability to the bacterium. Other traits concern resistance to abiotic stresses. Cells produce extracellular polymeric substances (EPS), which help to maintain a hydrated layer surrounding the bacteria and thus protect the cells from desiccation, and also help the bacteria to form aggregates. Biosurfactants are known to be produced by many epiphytic bacteria; these compounds increase wettability and also enhance leaching of substrates. Protection against radiation is achieved by pigment production, the activation of DNA repair enzymes and DNA protection during starvation protein (Dps). Other protective enzymes — catalases and superoxide dismutases — detoxify reactive oxygen species. In addition, bacteria might produce antibiotics to inhibit other microorganisms and, in contrast, might express efflux pumps to counteract toxic compounds of plant or microbial origin. In *Pseudomonas* spp., it has been found that flagellar motility contributes to epiphytic fitness, as does signalling via quorum sensing.

**Biochemical Reactions in the Phyllosphere**

Leaf surface microorganisms may perform an effective function in controlling the spread of air-borne pathogens inciting plant diseases. The presence of spores of a pathogen on the surface of leaves and pods results in the formation of a substance referred to as Phytoalexin’.  Alternatively, the phytoalexin may be normally present in plants and the concentration of such a substance may rise markedly in response to microbial infection. The term phytoalexin is derived from Greek phyto meaning plant and alexin meaning warding-off (prisoners cell) compound.

# Phytoalexin



Capsidiol is a phytoalexin produced by certain plants in response to pathogenic attack

**Phytoalexins** are antimicrobial and often antioxidative substances synthesized *de novo* by plants that accumulate rapidly at areas of pathogen infection. They are broad spectrum inhibitors and are chemically diverse with different types characteristic of particular plant species. Phytoalexins tend to fall into several classes including terpenoids, glycosteroids, alkaloids and natural phenols; however, researchers often find it convenient to extend the definition to include all phytochemicals that are part of the plant's defensive arsenal (source).

## Function

Phytoalexins produced in plants act as toxins to the attacking organism. They may puncture the cell wall, delay maturation, disrupt metabolism or prevent reproduction of the pathogen in question. Their importance in plant defense is indicated by an increase in susceptibility of plant tissue to infection when phytoalexin biosynthesis is inhibited. Mutants incapable of phytoalexin production exhibit more extensive pathogen colonization as compared to wild type. As such, host-specific pathogens capable of degrading phytoalexins are more virulent than those unable to do so.

When a plant cell recognizes particles from damaged **cells** or particles from the pathogen, the plant launches a two pronged (group of 2) resistance: a general short-term response and a delayed long-term specific response.

As part of the induced resistance, the short-term response, the plant deploys reactive oxygen species such as superoxide and hydrogen peroxide to kill invading cells. In pathogen interactions, the common short-term response is the hypersensitive response, in which cells surrounding the site of infection are signaled to undergo apoptosis (death due to DNA damage), or programmed cell death, in order to prevent the spread of the pathogen to the rest of the plant.

Long-term resistance, or systemic acquired resistance (SAR), involves communication of the damaged tissue with the rest of the plant using plant hormones such as jasmonic acid, ethylene, abscisic acid or salicylic acid. The reception of the signal leads to global changes within the plant, which induce genes that protect from further pathogen intrusion, including enzymes involved in the production of phytoalexins. Often, if jasmonates or ethylene (both gaseous hormones) is released from the wounded tissue, neighboring plants also manufacture phytoalexins in response. For herbivores, common vectors for disease, these and other wound response aromatics seem to act as a warning that the plant is no longer edible. Also, in accordance with the old adage, "an enemy of my enemy is my friend," the aromatics may alert natural enemies of the plant invaders to the presence thereof.

## Recent researches

[Allixin](http://en.wikipedia.org/wiki/Allixin) (3-hydroxy-5-methoxy-6-methyl-2-pentyl-4*H*-pyran-4-one), a non-sulfur-containing compound having a [γ-pyrone](http://en.wikipedia.org/wiki/Pyrone) skeleton structure, was the first compound isolated from [garlic](http://en.wikipedia.org/wiki/Garlic) as a phytoalexin, a product induced in plants by continuous [stress](http://en.wikipedia.org/wiki/Stress_%28mechanics%29). This compound has been shown to have unique biological properties, such as anti-oxidative effects, anti-microbial effects, anti-tumor promoting effects, inhibition of [aflatoxin](http://en.wikipedia.org/wiki/Aflatoxin) B2 [DNA](http://en.wikipedia.org/wiki/DNA) binding, and neurotrophic effects. Allixin showed an anti-tumor promoting effect in vivo, inhibiting skin [tumor](http://en.wikipedia.org/wiki/Tumor) formation by [TPA](http://en.wikipedia.org/wiki/12-O-Tetradecanoylphorbol-13-acetate) in [DMBA](http://en.wikipedia.org/wiki/7%2C12-Dimethylbenz%28a%29anthracene) initiated mice. Herein, allixin and/or its analogs may be expected useful compounds for cancer prevention or chemotherapy agents for other diseases.

## Role of natural phenols in the plant defence against fungal pathogens

Natural **phenols** play a role in the plant defence against fungal pathogens.

In *Vitis vinifera* grape, *trans*-resveratrol is a phytoalexin produced against the growth of fungal pathogens such as *Botrytis cinerea* and delta-viniferin is another grapevine phytoalexin produced following fungal infection by *Plasmopara viticola*. Pinosylvin is a pre-infectious stilbenoid toxin (i.e. synthesized prior to infection), contrary to phytoalexins which are synthesized during infection. It is present in the heartwood (wood at the centre of a tree) of *Pinaceae*. It is a fungitoxin protecting the wood from fungal infection.

Sakuranetin is a flavanone, a type of **flavonoid**. It can be found in [*Polymnia fruticosa*](http://en.wikipedia.org/wiki/Polymnia_fruticosa) and rice, where it acts as a phytoalexin against spore germination of *Pyricularia oryzae*. In *Sorghum*, the *SbF3'H2* gene, encoding a flavonoid 3'-hydroxylase, seems to be expressed in pathogen-specific 3-deoxyanthocyanidin phytoalexins synthesis, for example in *Sorghum-Colletotrichum* interactions.

6-Methoxymellein is a dihydroisocoumarin and a phytoalexin induced in carrot slices by UV-C, that allows resistance to *Botrytis cinerea* and other microorganisms.

**Danielone** is a phytoalexin found in the papaya fruit. This compound showed high antifungal activity against [*Colletotrichum gloesporioides*](http://en.wikipedia.org/wiki/Colletotrichum_gloesporioides), a pathogenic fungus of papaya.

Stilbenes are produced in *Eucalyptus sideroxylon* in case of pathogens attacks. Such compounds can be implied in the hypersensitive response of plants. High levels of polyphenols in some woods can explain their natural preservation against rot.

**Soil enzymes: Origin, activities and importance**

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Fig. Percentage distribution of different enzyme activities measured in studies concerning soil contamination with persistent organic pollutants (POPs)

**POPs**: Persistent organic pollutants (POPs) can occur in soils (both agricultural and natural soils) in significant amounts; these include phenolic compounds, mainly chlorophenols, and polyaromatic hydrocarbons (PAHs). Both of these groups include stable compounds that persist in the environment because of their structural properties. Many of these compounds are volatile and can circulate widely by a process known as the “grasshopper effect”. Once released by repeated (seasonal) processes of evaporation and deposition, these compounds are transported over long distances via the atmosphere. Moreover, these compounds are biochemically, chemically and physically recalcitrant, which enables them to accumulate in live organisms and thus reach humans through the food chain.

Chlorophenols are used as fungicides, bacteriocides and insecticides in the preservation of wood and leather, although they are also used as herbicides and as protective agents or antiseptics in plant production. Among the phenolic compounds, chlorophenols are common contaminants of soils and water. They are synthetic compounds that are obtained on commercial scale via the chlorination of phenols or by hydrolysis of chlorobenzenes. They can also be formed as intermediate products during the bleaching of paper pulp. Chlorophenols can also reach soils as degradation products of some herbicides and pesticides.

**FDA:** Fluorescein diacetate (FDA) hydrolysis assays can be used to measure [enzyme activity](https://en.wikipedia.org/wiki/Enzyme_activity) produced by microbes in a sample. This can be quantified using a spectrofluorometer. It is often used to measure activity in soil and compost samples.

Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system. They are important in catalysing several important reactions necessary for the life processes of microorganisms in soils and the stabilisation of soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling. These enzymes are constantly being synthesised, accumulated, inactivated and/or decomposed in the soil, hence playing an important role in agriculture and particularly in nutrients cycling. The activities of these enzymes in soils undergo complex biochemical processes consisting of integrated and ecologically-connected synthetic processes, and in the immobilisation and enzyme stability. In this regard, all soils contain a group of enzymes that determine soil metabolic processes which, in turn, depend on its physical, chemical, microbiological and biochemical properties. The enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amounts of organic matter content, composition and activity of its living organisms and intensity of the biological processes. In practice, the biochemical reactions are brought about largely through the catalytic contribution of enzymes and variable substrates that serve as energy sources for microorganisms. These enzymes may include amylase, arylsulphatases, β-glucosidase, cellulose, chitinase, dehydrogenase, phosphatase, protease and urease released from plants, animals, organic compounds and microorganisms and soils.

A better understanding of the role of these soil enzymes activity in the ecosystem will potentially provide a unique opportunity for an integrated biological assessment of soils due to their crucial role in several soil biological activities, their ease of measurement, and their rapid response to changes in soil management practices. Studies indicate that high enzyme activity signals **mineral element limitation** in the ecosystem. Although there have been extensive studies on soil enzymes, little has been reported on their roles in agricultural development. The roles of some of the enzymes’ activity and efficiency are reviewed for agricultural development.

**AMYLASE**

Amylase is a starch hydrolysing enzyme. It is known to be constituted by α-amylase and β-amylase. Studies have shown that α-amylases are synthesised by plants, animals and microorganisms, whereas, β-amylase is mainly synthesized by plants. This enzyme is widely distributed in plants and soils so it plays a significant role in the breakdown of starch. Research evidence suggests that several other enzymes are involved in the hydrolysis of starch, but of major importance are α-amylase which converts starch like substrates to glucose and/or oligosaccharides and β- amylase, which converts starch to maltose.

Studies have, however, indicated that the roles and activities of α-amylase and β-amylase enzymes may be influenced by different factors ranging from cultural practices, type of vegetation, environment and soil types. For example, plants may influence the amylase enzyme activities of soil by directly supplying enzymes from their residues or excreted compounds, or indirectly providing substrates for the synthetic activities of microorganisms.

**ARYLSULPHATASES**

It has been established that sulphur uptake in plants is in the form of inorganic sulphate (SO4) and its availability depends on its mineralisation or mobilisation from aromatic sulphate esters (RO-SO3-). This is due to the fact that certain proportions of sulphur in different soil profiles are bound into organic compounds and are indirectly available to plants. In this regard, its availability will depend on the extracellular hydrolysis of these aromatic sulphate esters or intracellular oxidation of soluble organic matter absorbed by the microorganisms to yield energy and carbon skeletons for biosynthesis by which some SO4-S are released as a by-product. All these processes are dependent on **arylsuphatases** enzymes. Arylsulphatases are typically widespread in nature as well as in soils. They are responsible for the hydrolysis of sulphate esters in the soil and are secreted by bacteria into the external environment as a response to sulphur limitation. Its occurrence in different soil systems is often correlated with microbial biomass and rate of S immobilisation. The role of this enzyme in the hydrolysis of aromatic sulphate esters (R-O-SO3-) to phenols (R-OH) and sulphate, or sulphate sulphur (SO4-2 or SO4-S) is shown in the following simple chemical equation:

 *Hydrolysis*

*R-O-SO3- R-OH + SO4-2*

 *Arylsulphatases*

Studies have shown that the release of sulphate from soluble and insoluble sulphate esters in the soil is affected by various environmental factors such as heavy metal pollution; pH changes in the soil solution; organic matter content and its type; the concentration of organic sulphate esters; the extent to which organic sulphate esters are protected against enzymatic hydrolysis such as sorption to particles surfaces in soils, and the activity persistence of extracellular arylsulphatases in the soil.

Considering the importance of S in plant nutrition, a better understanding of the role(s) of arylsulphatases in S mobilisation in agricultural soils is critical. So far, very little is known about specific microbial genera or species that play an important role in the soil organosulphur circle in which arylsulphatases is the key enzyme.

β**-GLUCOSIDASE**

β-glucosidase is a common and predominant enzyme in soils. It is named according to the type of bond that it hydrolyses. This enzyme plays an important role in soils because it is involved in catalysing the hydrolysis and biodegradation of various β-glucosides present in plant debris decomposing in the ecosystem. Its final product is glucose, an important C energy source of life to microbes in the soil. There is considerable evidence suggesting that a significant fraction of enzyme activity measured in soil originates from abiontic enzymes (enzymes of biological origin no longer associated with living cells) excreted into the soil solution or immobilised enzymes of microbial origin sorbed to clays or humic colloids.

β-glucosidase is characteristically useful as a soil quality indicator, and may give a reflection of past biological activity, the capacity of soil to stabilise the soil organic matter, and can be used to detect management effect on soils. This has greatly facilitated its adoption for soil quality testing. Generally, β-glucosidase activities can provide advanced evidence of changes in organic carbon long before it can be accurately measured by other routine methods. Several researchers have however also reported its phytopathological effects in the ecosystem. For example, some of the aglycons are known to be the precursors of the toxic substances which cause soil sickness where plants are grown as monocrops.

β-glucosidase enzyme is very sensitive to changes in pH, and soil management practices. The sensitivity to pH changes can be used as a good biochemical indicator for measuring ecological changes resulting from soil acidification in situations involving activities of this enzyme. β-glucosidase enzyme is also known to be inhibited by heavy metal contamination such as Cu and several others. For instance, studies have shown that plant debris did not decompose or show β-glucosidase activities when exposed to heavy metal polluted soils. Consequently, more understanding of the β-glucosidase enzyme activities and factors influencing them in the ecosystem may contribute significantly to soil health studies.

**CELLULASES**

Cellulose is the most abundant organic compound in the biosphere, comprising almost 50% of the biomass synthesised by photosynthetic fixation of CO2. Growth and survival of microorganisms important in most agricultural soils depends on the carbon source contained in the cellulose occurring in the soils. However, for carbon to be released as an energy source for use by the microorganisms, cellulose in plant debris has to be degraded into glucose, cellobiose and high molecular weight oligosaccharides by cellulases enzymes. Cellulases are a group of enzymes that catalyse the degradation of cellulose, polysaccharides build up of β-1, 4 linked glucose units. It has been reported that cellulases in soils are derived mainly from plant debris incorporated into the soil, and that a limited amount may also originate from fungi and bacteria in soils. Currently, it is generally accepted that the cellulases system comprises of three major types of enzymes. They include: endo-1, 4-β-glucanase which attacks the cellulose chains at random, exo-1, 4-β-glucanase which removes glucose or cellobiose from the non-reducing end of the cellulose chains, and β-D-glucosidase which hydrolyses cellobiose and other water soluble cellodextrins to glucose.

Demonstrating the effects of increasing concentrations of fungicides on cellulases activities, it has been shown that there is a decreasing effect with fungicides captan, cosan, thiram, zinels and sandolex. More recently, it has been reported that fungicides benlate, calixin and captan inhibit cellulose activity in *Fusarium monoliforme* isolates. Captatol inhibited cellulose activity in the sandy loam soil, and chlorothalonil showed a clear reduction in cellulase activity under flooded or non-flooded conditions.

Studies have shown that activities of cellulases in agricultural soils are affected by several factors. These include temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical structure of organic matter and its location in the soil profile horizon, quality of organic matter/plant debris and soil mineral elements and the trace elements from fungicides. Recent research reported a significantly more stimulatory effect of cellulases in black soil than red soil. Several mechanisms have been proposed in the degradation of cellulose by cellulases. For instance, chitin in the presence of cellulose induces the synthesis of chitinase and other cell wall lytic enzymes which promote the release of the intramural β-glucosidase into the medium. All these findings suggest that activities of cellulases can be used to give preliminary indication of some of the physic-chemical properties of soil, thus, easing agricultural soil management strategies. Since cellulases enzymes play an important role in global recycling of the most abundant polymer, cellulose in nature, it would be of critical importance to understand this enzyme better so that it may be used more regularly as a predictive tool in our soil fertility programmes.

**CHITINASE**

Chitinase or chitinolytic enzymes are key enzymes responsible for the degradation and hydrolysis of chitin (poly β-1-4-(2-ncetamido-2-deoxy)-D-glucoside). They are also considered as the major structural component of many fungal cell walls that use the hyperparasitism mechanisms against pests/pathogen attack. These biological agents also reduce disease producing agents by using other mechanisms such as antibiosis or competition mechanisms. This agriculturally important enzyme is produced or released by various organisms including plants and microorganisms. For example, in plants, the chitinase enzyme is induced and accumulated in response to microbial infections and it is thought to be involved in the defence of plants against pathogen infections. Its presence in different forms in the ecosystem has demonstrated its effectiveness in the control of soil-borne diseases such as *Sclerotium rolfsii* and *Rhizoctonia solani* in beans and cotton, respectively. Biological control of damping off caused by *R. solani* was achieved by applying antagonistic fungi and bacteria isolated from coastal soils with chitinase activities. One of the mechanisms proposed involves lytic enzymes that cause the degradation of cell walls of pathogenic fungi.

**DEHYDROGENASE**

The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils. This enzyme is considered to exist as an integral part of intact cells but does not accumulate extracellularly in the soil. Dehydrogenase enzyme is known to oxidise soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are part of respiration pathways of soil microorganisms and are closely related to the type of soil and soil air-water conditions. Since these processes are part of respiration pathways of soil microorganisms, studies on the activities of dehydrogenase enzyme in the soil is very important as it may give indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility.

With regard to soil air-water relationships, studies have shown that dehydrogenase enzyme was greater in flooded compared to non-flooded soil. The increase in this enzyme after flooding was also related to decreased redox potential. One study suggested that soil water content and temperature influence dehydrogenase activity indirectly by affecting the soil redox status.

After flooding the soil, oxygen present is rapidly exhausted so that a shift of the activity from aerobic to anaerobic microorganisms takes place. Such redox transformations are closely connected with respiration activity of soil microorganisms. They may serve as indicators of the microbiological redox systems in soils and can be considered a possible measure of microbial oxidative activity. The relationship between dehydrogenase activity and redox potential (Eh) as well as Fe2+ content may also be used to illustrate the reactions of soil microorganisms to the changes in soil environment. For instance, lack of oxygen may trigger facultative anaerobes to initiate metabolic processes involving dehydrogenase activities and the use of Fe (III) forms as terminal electron acceptors, a process that may affect iron availability to plants in the ecosystem. Some studies have shown that reducing conditions in the soil were associated with high Fe2+ concentration in the soil solution and a significant increase of extra plasmatic Fe in roots of maize due to intense stimulation of microbial growth and dehydrogenase activities in the ecosystem.

Additionally, dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil, as well as a direct measure of soil microbial activity. It can also indicate the type and significance of pollution in soils. For example, dehydrogenase enzyme is high in soils polluted with pulp and paper mill effluents but low in soils polluted with fly ash. Similarly, higher activities of dehydrogenases have been reported at low doses of pesticides, and lower activities of the enzyme at higher doses of pesticides. As most areas of the world are often polluted by different industrial bio-chemical products, better understanding of the role of this enzyme in environmental science will open greater possibilities of using it as a diagnostic tool for better ecosystem assessment and amelioration.

**PHOSPHATASES**

Phosphatases are a broad group of enzymes that are capable of catalysing hydrolysis of esters and anhydrides of phosphoric acid. In soil ecosystems, these enzymes are believed to play critical roles in P cycles as evidence shows that they are correlated to P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system.

Land plants have evolved many morphological and enzymatic adaptations to tolerate low phosphate availability. This includes transcription activity of acid phosphatases, which tend to increase with high P stress. For example, when there is a signal indicating P deficiency in the soil, acid phosphatase secretion from plant roots is increased to enhance the solubilisation and remobilisation of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions.

The amount of acid phosphatase exuded by plant roots has been shown to differ between crop species and varieties, as well as crop management practices. For instance, research has shown that legumes secrete more phosphatise enzymes than cereal. This may probably be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals. For example: chickpea roots are able to secrete greater amounts of acid phosphatase than maize.

The ability to solubilise soil mineral elements by these phosphomonoesteraces is expected to be a higher in biologically-managed systems because of a higher quantity of organic C found in those systems. In fact, the activity of acid and alkaline phosphatases was found to correlate with organic matter in various studies. Another factor that influences the rate of synthesis, release and stability of this enzyme is the soil pH. For example, phosphomonoesteraces inducibility and their exudation intensity by plant roots and microorganisms are determined by their orthophosphate need, which is in turn affected by soil pH. It is, therefore, anticipated that management practices that induce P stress in the rhizosphere may also affect the secretion of these enzymes in the ecosystem.

**PROTEASE**

Proteases in soil play a significant role in N mineralisation, an important process regulating the amount of plant available N and plant growth. This enzyme in the soil is generally associated with inorganic and organic colloids. Protease activities have been reported to occur partly in soil as a humocarbohydrate complex from arable soil; from solid municipal waste compost, and from forest or permanent grassland soils. The amount of this extracellular enzyme activity may be indicative not only of the biological capacity of soil for the enzymatic conversion of the substrate, which is independent of the extent of microbial activity, but might also have an important role in the ecology of microorganisms in the ecosystem.

Protease activities are affected by several biotic and abiotic factors. For example, low concentrations of neutralised soil humic acids (l-100 pg/ml) inhibit some and stimulate other protease activity by mechanisms involving primarily humic acid carboxyl groups. The enzyme pronase is inhibited irrespective of the charge of the substrate hydrolysed, suggesting that decreased activity results from humic acid combining with enzyme rather than with substrate. Furthermore, quantitative considerations of the effects of humic acid and substrate concentrations on pronase hydrolysis of carbobenzoxy-glycyl leucine indicates that inhibition is not due to the combination of humic acid and substrate anions.

**UREASE**

Urease enzyme is responsible for the hydrolysis of urea fertiliser applied to the soil into NH3 and CO2 with the concomitant rise in soil pH. This, in turn, results in a rapid N loss to the atmosphere through NH3 volatilisation. Due to this role, urease activities in soils have received a lot of attention since a process considered vital in the regulation of N supply to plants after urea fertilisation.

Often, urea is the main source of N in many crops including flooded or irrigated rice and maize in many parts of Africa and Asia. Despite the importance of this fertiliser, its efficiency has been reported as low due to substantial N lost to the atmosphere through volatilisation, a process mediated by the urease enzyme.

Soil urease originates mainly from plants and microorganisms found as both intra- and extra-cellular enzymes. The stability of this enzyme in the system is affected by several factors. For example, studies have shown that extracellular urease associated with soil organo-mineral complexes is more stable than urease in the soil solution and those humus-urease complexes extracted from soil are highly resistant to denaturing agents such as extreme temperatures and proteolytic attack. On the other hand, urease extracted from plants or microorganisms is rapidly degraded in soil by proteolytic enzymes. This suggests that a significant fraction of ureolytic activity in soil is carried out by extracellular urease, which is stabilised by immobilisation on organic and mineral soil colloids.

Urease activity in soils is influenced by many factors. These include cropping history, organic matter content of the soil, soil depth, soil amendments, heavy metals, and environmental factors such as temperatures. For example, studies have shown that urease was very sensitive to toxic concentrations of heavy metals. Other studies with soil samples taken from horizons of different soil profiles revealed decreased activities with increased soil depth. The differences were attributed to decreases in soil organic matter content with depth. The effect of temperature on urea hydrolysis has received considerable research attention. Generally, urease activity increases with increasing temperature. It is suggested that higher temperatures increase the activity coefficient of this enzyme. Therefore, it is recommended that urea be applied at times of the day when temperatures are low. This is because during such times the activation energy is low, thus, resulting in minimum loss of N by the volatilisation process.

Since urease plays a vital role in the hydrolysis of urea fertiliser, it is important to uncover other unknown factors that may reduce the efficiency of this enzyme in the ecosystem. A better understanding of this enzyme would provide more effective ways of managing urea fertiliser especially in high rainfall areas, flooded soils and irrigated lands as well as where urea fertiliser is vulnerable to urease enzyme.

**CONCLUSION**

Understanding other possible roles of soil enzymes is vital to soil health and fertility management in ecosystems. These enzymes may have significant effects on soil biology, environmental management, growth and nutrient uptake in plants growing in ecosystems. Their activities may, however, be influenced by unknown cultural management practices.

**Factors Affecting Distribution, Activity and Population of Soil Microorganisms**

Soil microorganisms (Flora and Fauna), just like higher plants depends entirely on soil for their nutrition, growth and activity. The major soil factors which influence the microbial population, distribution and their activity in the soil are

1. Soil fertility 2. Cultural practices 3. Soil moisture 4. Soil temperature 5. Soil aeration 6. Light 7. Soil pH (H+ concentration) 8. Organic matter 9. Food and energy supply 10. Nature of soil and 11. Microbial associations.

All these factors play a great role in determining not only the number and type of organism but also their activities. Variations in any one or more of these factors may lead to the changes in the activity of the organisms which ultimately affect the soil fertility level. Brief account of all these factors influencing soil microorganisms and their activities are discussed in following paragraphs.

**1. Cultural practices (Tillage):** Cultural practices viz. cultivation, crop rotation, application of manures and fertilizers, liming and gypsum application, pesticide/fungicide and weedicide application have their effect on soil organism. Ploughing and tillage operations facilitate aeration in soil and exposure of soil to sunshine and thereby increase the biological activity of organisms, particularly of bacteria. Crop rotation with legume maintains the favorable microbial population balance, particularly of N2 fixing bacteria and thereby improve soil fertility.

Liming of acid soils increases activity of bacteria and actinomycetes and lowers the fungal population. Fertilizers and manures applied to the soil for increased crop production, supply food and nutrition not only to the crops but also to microorganisms in soil and thereby proliferate the activity of microbes.

Foliar or soil application of different chemicals (pesticides, fungicides, nematicides etc.) in agriculture are either degraded by the soil organisms or are liable to leave toxic residues in soil which are hazardous to cause profound reduction in the  normal microbial activity in the soil.

**2. Soil fertility:**Fertility level of the soil has a great influence on the microbial population and their activity in soil. The availability of N, P and K required for plants as well as microbes in soil determines the fertility level of soil. On the other hand soil micro flora has greater influence on the soil fertility level.

**3. Soil moisture:**It is one of the important factors influencing the microbial population and their activity in soil. Water (soil moisture) is useful to the microorganisms in two ways i.e. it serve as source of nutrients and supplies hydrogen / oxygen to the organisms and it serve as solvent and carrier of other food nutrients to the microorganisms. Microbial activity and population proliferate best in the moisture range of 20% to 60%. Under excess moisture conditions / water logged conditions due to lack of soil aeration (Oxygen) anaerobic microflora become active and the aerobes get suppressed. While in the absence of adequate moisture in soil, some of microbes die out due to tissue dehydration and some of them change their forms into resting stages spores or cysts and tide over adverse conditions. Therefore optimum soil moisture (range 20 to 60 %) must be there for better population and activity of microbes in soil.

**4.  Soil temperature:**Next to moisture, temperature is the most important environmental factor influencing the biological physical and chemical processes and of microbes, microbial activity and population in soil. Though microorganisms can tolerate extreme temperature (such as - 60 ° or + 60 u) conditions, but the optimum temperature range at which soil microorganisms can grow and function actively is rather narrow.

Depending upon the temperature range at which microorganisms can grow and function, are divided into three groups i.e. psychrophiles (growing at low temperature below 10 °C), mesophiles (growing well in the temp range of 20 ° C to 45° C) and thermopiles (can tolerate temperature above 45° C and optimum 45-60°C).

Most of the soil microorganisms are mesophilic (25 to 40 °C) and optimum temperature for most mesophiles is 37° C. True psychrophiles are almost absent in soil, and thermopiles though present in soil behaves like mesophiles. True thermopiles are more abundant in decaying manure and compost heaps where high temperature prevails.

Seasonal changes in soil temperature affect microbial population and their activity especially in temperate regions. In winter, when temperature is low (below 50° C ), the number and activity of microorganisms falls down, and as the soils warms up in spring, they increases in number as well as activity. In general, population and activities of soil microorganisms are the highest in spring and lowest in winter season.

**5.  Soil air (Aeration):**For the growth of microorganisms better aeration (oxygen and sometimes CO2) in the soil is essential. Microbes consume oxygen from soil air and gives out carbon dioxide. Activities of soil microbes is often measured in terms of the amount of oxygen absorbed or amount of CO2 evolved by the organisms in the soil environment. Under high soil moisture level / water logged conditions, gaseous exchange is hindered and the accumulation of CO2 occurs in soil air which is toxic to microbes. Depending upon oxygen requirements, soil microorganisms are grouped into categories viz aerobic (require oxygen for like processes), anaerobic (do not require oxygen) and microaerophilic (requiring low concentration / level of oxygen).

**6. Light:** Direct sunlight is highly injurious to most of the microorganisms except algae. Therefore upper portion of the surface soil a centimeter or less is usually sterile or devoid of microorganisms. Effect of sunlight is due to heating and increase in temperature (more than 45°)

**7. Soil Reaction / Soil PH:**Soil reaction has a definite influence / effect on quantitative and qualitative composite on of soil microbes. Most of the soil bacteria, blue-green algae, diatoms and protozoa prefer a neutral or slightly alkaline reaction between pH 4.5 and 8.0 and fungi grow in acidic reaction between PH 4.5 and 6.5 while actinomycetes prefer slightly alkaline soil reactions. Soil reactions also influence the type of the bacteria present in soil. For example nitrifying bacteria (Nitrosomonas and Nitrobacter)and diazotrophs like Azotobacterare absent totally or inactive in acid soils, while diazotrophs like Beijerinckia, Derxia,and sulphur oxidizing bacteria like Thiobacillus thiooxidansare active in acidic soils.

**8. Soil Organic Matter:**The organic matter in soil being the chief source of energy and food for most of the soil organisms, it has great influence on the microbial population. Organic matter influences directly or indirectly on the population and activity of soil microorganisms. It influences the structure and texture of soil and thereby activity of the microorganisms.

**9.  Food and energy supply:**Almost all microorganisms obtain their food and energy from the plant residues or organic matter / substances added to the soil. Energy is required for the metabolic activities of microorganisms. The heterotrophs utilize the energy liberated during the oxidation of complex organic compounds in soil, while autotrophs meet their energy requirement form oxidation of simple inorganic compounds (chemoautotroph) or from solar radiation (photoautotroph). Thus, the source of food and energy rich material is essential for the microbial activity in soil. The organic matter, therefore serves both as a source of food nutrients as well as energy required by the soil organisms.

**10. Nature of Soil:**The physical, chemical and physico-chemical nature of soil and its nutrient status influence the microbial population both quantitatively and qualitatively. The chemical nature of soil has considerable effect on microbial population in soil. The soils in good physical condition have better aeration and moisture content which is essential for optimum microbial activity. Similarly nutrients (macro and micro) and organic constituents of humus are responsible for absence or presence of certain type of microorganisms and their activity. For example activity and presence of nitrogen fixing bacteria is greatly influenced by the availability of molybdenum and absence of available phosphate restricts the growth of Azotobacter.

**11.  Microbial associations / interactions:**Microorganisms interact with each other giving rise to antagonistic or symbiotic interactions. The association existing between one organism and another whether of symbiotic or antagonistic influences the population and activity of soil microbes to a great extent. The predatory habit of protozoa and some mycobacteria which feed on bacteria may suppress or eliminate certain bacteria. On the other hand, the activities of some of the microorganisms are beneficial to each other. For instance organic acids liberated by fungi, increase in oxygen by the activity of algae, change in soil reaction etc. favors the activity or bacteria and other organisms in soil.

**12.   Root Exudates:**In the soil where plants are growing the root exudates also affects the distribution, density and activity of soil microorganism. Root exudates and sloughed off material of root surfaces provide an abundant source of energy and nutrients and thus directly or indirectly influence the quality as well as quantity of microorganisms in the rhizosphere region. Root exudates contain sugars, organic acids, amino acids, sterols, vitamins and other growth factors which have the profound effect on soil microbes.