CHAPTER-4

EUKARYOTIC ALGAE AND CYANOBACTERIA

Soil algae are simple photoautotrophic organisms that lack tissue differentiation, but they vary greatly in morphology, physiology, reproduction, and habitat. Although considerable number of algae is present at the surface and within the surface layers of most soils, there is a general lack of awareness of their presence. Hence, the algae have been much less studied than nonphotosynthetic microorganisms. This lack of attention has fostered the impression that they are not an important component of the community of soil microorganisms, even though algae may be the only primary producers present in certain ecosystems. The study of soil algae can be traced to the beginning of the nineteenth century. Beijerinck first reported the isolation of soil algae in 1893.

Certain species of soil algae are potentially useful for food or energy during space travel. Although the production of phototrophic algae on a large scale is uncommon, some have suggested that algae could be a major source of future dietary protein, both in space and on earth. Indeed, some (e.g. *Spirulina*) are already sold as dietary supplements. Hydrogen production by immobilized cyanobacteria also is being investigated as a source of nonpolluting fuel. In addition, algae could remove nutrients from wastewater and replenish oxygen in closed life-support system.

CLASSIFICATION

There are two major groups broadly defined as algae:

1. **Eukaryotic algae** are part of plant kingdom.
2. **Prokaryotic cyanobacteria** (formerly known as blue-green algae) are part of the Bacteria

Following are the most common soil algae

- Cyanobacteria (class *Cyanophyceae*)
- Green algae (class *Chlorophyceae*)
- Diatoms (class *Bacillariophyceae*)
- Yello-green algae (class *Xanthophyceae*)

The less frequent soil algae include

- Euglenoid (class *Euglenophyceae*)
- Red algae (class *Rhodophyceae*)

Early classification schemes relied heavily on the presence of specific photosynthetic and accessory pigments. Current systems of classification additionally incorporate information about cell-wall constituents, cellular organization, flagellation, and molecular biology.

Major Groups Found in Soil:

Cyanobacteria:

The cyanobacteria are widely distributed. In addition to soil, the terrestrial species may also be found on plants, rocks, and even animals. All species belonging to this group are unicellular or filamentous; cells frequently remain together, surrounded by a gelatinous material. At the subcellular level, the cyanobacteria are morphologically and physiologically similar to bacteria. Their cell walls show some similarity to those of bacteria. As would be expected, cyanobacterial DNA is not separated from the rest of the cytoplasm by a nuclear membrane; hence there is no distinct nucleus. However, many species may have a relatively dense mass of material called the *central body*. Cyanobacteria do not have chloroplasts; their photosynthetic pigments are usually associated with membranous layers called *lamellae* and appear uniformly distributed throughout the cytoplasm. The cyanobacteria contain chlorophyll a, but not chlorophyll b; however, the dominant pigment is the blue-coloured *phycocyanin.* The storage product in cyanobacteria is starch like but somewhat different from that of higher plants. Some species store food reserves as oils. Reproduction is by simple cell division without mitosis. The cyanobacteria produce several different types of immobile spores. They are usually found within a filament or trichome, arising from a vegetative cell. They form a thick wall after being filled with food reserves. Many filamentous species can form spore like cells called *heterocyst*, which are involved in N_2 fixation.

Green algae:

In contrast to the cyanobacteria, considerable cellular organization exists in the eukaryotic green algae. Their organization and physiology closely resemble that of higher plants. In general, the cell walls of eukaryotic algae are similar to those of higher plant; their DNA is localized within minutely perforated nuclear membranes, and the photosynthetic apparatus, including the pigments, is contained in chloroplasts. Green algae possess chlorophyll a as the predominant pigment. In addition to the nucleus, eukaryotic cells may also contain organelles, including vacuoles, flagella, Golgi bodies, and mitochondria.

Diatoms:

Diatoms live in fresh and salt water and in the soil. Terrestrial diatoms are generally smaller than aquatic species, and most are capable of movement. The diatoms are usually unicellular but may occur in filamentous colonies or as branched or unusual clusters. The cell wall or *frustule* is composed of two slightly overlapping valves. The cytoplasm contains a single nucleus and one to many plastids. The chloroplasts contain chlorophyll a and c. The carotenoid and golden brown fucoxanthin give the cells their characteristic color varying from green or yellow to golden-brown. The normal method of reproduction is asexual by division of one cell into two. The storage product in the diatoms is chrysolaminarin, a β-1,3 linked glucan. Diatoms form resting spores with thick, ornamented walls. In addition, they can form resting cells, distinct from the spores that are morphologically similar to vegetative cells but lack a protective layer.

Yellow-green algae:

The yellow-green algae can be motile, with separate forward-directed and posteriorly directed flagella. Their chloroplasts contain chlorophyll a but lack fucoxanthin; other carotenoid pigments provide the characteristic yellow-green coloration of this group. The cell walls of the yellow-green algae are usually composed of cellulose and consist of two overlapping halves. The principal storage product is probably a β-1,3 linked glucan, but research suggests that lipids are also important for food storage. Members of this class usually multiply asexually by fragmentation and motile or nonmotile spores. Sexual reproduction has been found in *Botrydium* and *Vaucberia*. They have the ability to form resting spores.

MORPHOLOGY AND REPRODUCTION

Soil algae are simple, undifferentiated organisms. They may occur as unicellular types or as cell aggregates with cell aggregates ranging from filamentous to more complex colonial species. Individual algal cells may also differ greatly in size and shape. The cyanobacteria are similar in size to other prokaryotes and commonly have cellular volumes ranging from 5 to 50 μm^3, although volumes from 0.1 to 5,000 μm^3 are also known. The unicellular eukaryotic algae are somewhat larger and have a normal range of 5,000 to 15,000 μm^3, with extremes of 5 to 100,000 μm^3. Rudimentary colonies are formed when several generations of cells remain attached after cell division. Other groupings may take the form of branched or unbranched filaments called *trichomes*. Although these organisms are considered simple, they possess great diversity.

Many soil algae are able to form spores. These spores are a means of carrying the species over periods of unfavourable conditions rather than a means of reproduction. They can be formed either sexually or asexually. Some species may form thick-walled resting stages, which are usually morphologically specialized structures. However, formation of resting spores may involve primarily physiological changes, including decreased metabolic activity or the production of protective mucilage.

Algae demonstrate as much variation in reproduction as they do in morphology; vegetative, asexual, and sexual processes are all present. Reproduction in the eukaryotic algae is more varied than in other groups. Practically all the green algae include some form of sexual reproduction. Reproduction in the diatoms and yellow-green algae is usually asexual by simple cell division. In the euglenoids, the nucleus commonly divides mitotically, and reproduction is by longitudinal cell division.

PHYSIOLOGY

Photosynthesis:

Photosynthesis is the most important biochemical reaction on earth. All our food and biological fuels are products of photosynthesis, a complex reaction in which light energy is used to convert carbon dioxide and water to carbohydrates and molecular oxygen. The biochemistry of both prokaryotic and eukaryotic photosynthesis is very similar to that of higher plants. In fact, the most common pathway of carbon dioxide fixation (the C-3 pathway or Calvin cycle) was first described in the green algae.

Photosynthesis in eukaryotic algae and higher plants is carried out in subcellular organelles called *chloroplasts*. The chloroplast, which is bounded by a double membrane, contains both the photosynthetic pigments and the electron-transport systems necessary for photophosphorylation. Chloroplasts also contain the enzymes for carbon assimilation and, therefore, are complete units of photosynthetic function. In contrast, the prokaryotic cyanobacteria do not have chloroplasts. Instead, the photochemical reactions are found in the *lamellae* containing photosynthetic pigments. This arrangement is structurally and functionally simpler than the chloroplasts of the eukaryotic algae. The enzymes used in the dark reactions of photosynthesis are found in the cytoplasm.

Three main classes of pigments are associated with the photosynthetic apparatus: *chlorophylls*, *carotenoids*, and *phycobilin*s. The chlorophylls and carotenoids are always present, but phycobilins are limited to a few groups of algae.

Dinitrogen Fixation:

The reduction or fixation of atmospheric dinitrogen (N_2) to ammonia (NH_3) is an important process in the nitrogen cycle. The largest contribution to biological N_2 fixation from free living organisms is made by cyanobacteria in the tropics. For example, in rice paddy agroecosystems, the cyanobacteria that grow with rice plants contribute a substantial amount of nitrogen to the crop.

The ability of N_2-fixing cyanobacteria to withstand adverse conditions accounts, in part, for their wide distribution and emphasizes their ecological importance. Dinitrogen fixation by cyanobacteria is sensitive to pH and decreases markedly below a pH of about 6.6. Unfavourable growth conditions can promote excretion of fixed nitrogen from the cell as inorganic (NH_3 or NH_4^+) or organic nitrogen and can represent a significant nutrient and energy drain.

The enzymes responsible for catalyzing biological nitrogen fixation are strongly inhibited by oxygen. The N_2-fixing cyanobacteria have several mechanisms to protect these enzymes from inactivation by oxygen. These mechanisms will be elaborated while discussing biological nitrogen fixation in a separate chapter.

Some N_2-fixing cyanobacteria form symbiotic associations of considerable economic importance. For example, *Azolla* is a small aquatic fern with a wide distribution that, together with the N_2-fixing cyanobacterium *Anabaena Azollae*, can contribute nitrogen to rice production. Similarly, *lichen* (symbiotic association between a fungus and cyanobacteria) may also contribute to nitrogen additions.

ECOLOGY

Distribution:

Soil algae exist from polar to tropical regions, although their abundance is usually greatest in the tropics. Algal communities in Antarctic soils consist of restricted number of taxa, and a given location may be dominated by only a few species. In contrast, hot and dry desert soils may have algae and lichens as the dominant microflora. Terrestrial algae can be found on and in the soil and exposed rocks, mud, sand, and snow. Algae can even be attached to buildings, plants, and animals. There are reports of roof and building discolouration caused by cyanobacteria and green algae.

Under both stressful and hospitable conditions, a considerable population of algae is present at the surface and within the subsurface layers of most soils. As many as 10^8 algae g^{-1} of soil have been found, although populations between 10^3 and 10^4 g^{-1} are more common-several orders of magnitude less than for bacteria and fungi, but similar to levels commonly observed for protozoa.

Algal numbers usually decreases with increasing depth and decreasing light intensity. Substantial populations of soil algae may be found in forest soils, although low light intensity may be a limiting factor to algal growth in these habitats. Algal numbers are greatest in the upper 30 cm of soil, even though some algae may occur to a depth of two meters. However, there is no evidence that algae present deep in soils are metabolically active.

Spatial distribution of algae can vary widely. Algal populations at sites as little as 10 cm apart have been shown to differ, both quantitatively and qualitatively, due to fluctuations in moisture, pH, nutrient availability, and light intensity. Seasonal changes in the soil algae are generally quantitative and result from fluctuations in soil moisture.

Terrestrial Colonization:

Human activities, along with climatological and geological events, are constantly changing the soil environment. However, no soil remains devoid of algae for much longer than the first gust of wind. Algae are also transported to new environments by water movement, including floods and runoff, and humans and other animals. Algae are pioneer colonizers in barren zones, such as volcanic lava and ash field, and are important in early stages of soil formation. They have an important role in plant community succession by providing primary production, biological weathering of minerals, N_2 fixation, and stabilization of soil aggregates.

Microscopic algae are found in the atmosphere in numbers up to a few hundred m^{-3} and represent a source of inoculum for terrestrial colonization. Soil algae may contribute to this airborne population when the soil they inhabit is carried into the atmosphere and widely distributed. Most airborne algae have been identified as green algae; cyanobacteria and diatoms are far less numerous. Algae may settle from the air or be removed by rainfall, and numerous algae have been found in rainwater.

Environmental Interactions:

Soil algae are subject to many environmental stresses, both biotic and abiotic. Solar radiation, temperature, and particularly water stress are important abiotic factors governing their distribution, metabolism, and life strategies. Algae are not a static soil component; they are constantly interacting with other biota and their physical and chemical environment. Some soil algae are capable of life in very hostile environments.

Moisture:

Algae are most abundant when soil is moist or waterlogged for extended period of time. Although drying conditions are stressful, algae can tolerate desiccation. Many soil algae, especially the cyanobacteria, have well-developed mechanisms for drought tolerance. Tolerance to desiccation often depends on the particular combination of physical factors present and the type of organism undergoing the stress. Most soil algae, especially the green algae and cyanobacteria, possess thick mucilaginous sheaths, which protect them against desiccation. Some soil algae form spores, whereas others can tolerate desiccation without apparent special morphological adaptations. Soil diatoms can survive adverse conditions through the storage of energy–rich oils, the buildup of inner plates, and reduction in cell size. To tolerate desiccation and heat, many cyanobacteria produce *akinetes* (thick-walled spores), which are resistant to adverse conditions. The duration of desiccation may influence the size and composition of algal populations.

Light:

Algae, being phototrophic organisms, are most common in the upper level of the soil. This layer may be referred to as *photic zone*. It is difficult to assign an exact depth to the photic zone, as light penetration of the soil varies with soil constituents that either reflect (e.g., residue cover) or absorb (e.g., soil organic matter) light. In addition, a physical disruption of the soil, such as tillage, can alter the photic zone. Compared to the eukaryotic soil algae, the cyanobacterial species are repressed by high light intensities. This constraint to their development allows diatoms and unicellular green algae to develop first after a desiccated soil has been rewetted. Cyanobacteria appear when plant cover is sufficiently dense to protect them from high light intensities. Some algae are common in caves, where light intensities are very low. Although thee reports of heterotrophic growth in the absence of light among the green algae, cyanobacteria, and diatoms, it is unlikely that these algae can compete successfully with common soil heterotrophs.

Temperature:

Soil algae are metabolically active over a wide range of temperatures. Most cyanobacteria prefer temperatures of 30^0 to 35^0C, and their population may increase relative to eukaryotic soil algae as the temperature increases. On the other hand, the algae of frozen habitat have also been reported which occur characteristically on the surface of snow.

Acidity:

The direct effect of pH on the soil algae is difficult to evaluate because pH is correlated with other factors. Nearly neutral or slightly alkaline soils support the most varied algal flora. The cyanobacteria, especially the filamentous forms, are most common in alkaline or neutral soils, but they have also been found in acidic soils. Cyanobacteria do not exist at pH levels below 4, although eukaryotic algae may exist and even grow profusely at that pH. The liming of soils may stimulate cyanobacterial growth by increasing the pH. Although the green algae may be found in soils of a wide pH range, they tend to be the most abundant group in acid habitats, probably because of an absence of competition. *Cyanidium caldarium* will grow in hot and extremely acid soils and is the only known photosynthetic organism living at a pH lower tan 5 and a temperature greater 40^0C. Diatoms are generally present in neutral or slightly alkaline soils. Some red algae seem to tolerate adverse habitats, such as extremely acid, polluted, or ammonium-rich soils.

Nutrients:

The greater majority of soil algae are obligate photoautotrophs; that is, they use light energy to manufacture all of their organic compounds from inorganic precursors. Thus, they require only water, solar radiation, carbon dioxide, and essential inorganic nutrients for growth. The nutritional requirements for macroelements and microelements are similar to those of higher plants. However, some hardly noticeable differences are found; for example, many cyanobacteria have a substantial physiological requirement for phosphorous. The availability of nutrients has an important influence on algal diversity and biomass. The algae are aerobic organisms and require oxygen to support their respiratory functions, including oxidative phosphorylation.

The nutritional relationships of soil algae to other organisms have not been widely explored. The soil algae are primary producers of organic compounds and thus play a central role as the base of the food chain. A number of soil algae belong to other nutritional categories, including photoheterotrophs, which utilize solar radiation as an energy source but cannot synthesize all necessary organic compounds from inorganic precursors. While heterotrophic activity is rare, some green algae in the soil can use organic complexes produced by the degradative activities of other microorganisms.

Pesticides and pollutants:

The soil algae are affected by a wide variety of herbicides, fungicides, pollutants and soil fumigants. The photosystem-uncoupling herbicides (e.g., diuron, atrazine, and simazine) are generally the most harmful. Various genera react differently to certain pesticides, and the pesticide regimen of cultivated soils may determine microfloral composition. Cyanobacteria appear to be less sensitive than eukaryotic algae and crop plants to many agrochemicals, and their growth is sometimes stimulated because of inhibition of green algal competitors by certain agrochemicals. Algae can be useful indicators of pesticide residues in the soil. Changes in their populations or metabolic activity may provide information on the accumulation of such chemicals and their possible detrimental effects on crop plants.

Biotic Interactions:

Interactions of the soil algae with other soil organisms are not well understood but range from antagonistic relations with other soil microbes to symbiotic associations with plants and fungi. Soil algae are a food source for grazing soil animals, including protozoa and nematodes. Soil algae may affect higher plants through the production of plant growth regulators. Several species produce natural toxins, including *cyanobacterin* and other antibiotics, which inhibit the growth of competing cyanobacteria and eukaryotic algae. For example, *Microcystis* spp. produces the toxin *microcystin* which inhibits the growth of green algae and diatoms. Growth-promoting or inhibitory substances may play an important role in the succession of algal species in the soil. Only rarely are the soil algae considered pathogenic. However, when algae become airborne, they can cause allergic reactions.

Beneficial interactions:

Soil algae may enter into relationships with other microorganisms; for example, many soil protozoa contain *endosymbiotic* algae. Algae can also enter into associations with numerous higher plant species. The N_2-fixing cyanobacteria may be *endophytic* within certain liverworts, the aquatic fern *Azolla, Gunnera* spp., and in the roots of *cycads* (one of the most primitive of living seed plants). The cyanobacteria provide the plant with fixed nitrogen, while the algae are protected from environmental stresses by the association or symbiosis.

An alga and a fungus can combine to form a *lichen*, a symbiotic association in which the two organisms are intergrown to form a single thallus. The photosynthetic partner (*phycobiont*) may be either an eukaryote, generally a green alga, or a prokaryotic cyanobacterium, usually of the genus *Nostoc*. The fungal member is usually an ascomycete, less often a basidiomycete. There are roughly 18,000 species of lichenized ascomycetes. In the symbiosis, the phycobiont captures light energy for carbon fixation and, if the phycobiont is a cyanobacterium, it may also fix atmospheric N_2. In return for the fixed carbon and nitrogen, the fungal partner (*mycobiont*) is believed to furnish mineral nutrients and help to regulate the water and light environment of its symbionts. The association may allow the survival and even promote the growth of the two partners in severe environments where neither could survive alone. As the lichens can withstand considerable water stress, they play an important role in arid ecosystem.

Detrimental interaction:

The soil algae must compete with other photosynthetic organisms for light and with soil microorganisms in general for inorganic nutrients. When present, higher plants also compete with algae for moisture and nutrients. Several Cyanobacteria species produce natural toxins, which inhibit other Cyanobacteria and eukaryotic algae. Algae parasitic on plants are known only among the green algae, and, because they mainly occur on noncultivated plants, they are generally overlooked.

All the soil algae are subject to parasitism and predation. The inoculation of soil with N_2-fixing cyanobacteria is rarely successful because their populations are rapidly reduced, apparently because of grazing by soil animals. Viruses are widespread and highly specific in their infection abilities; cyanophages are the viruses specific to the cyanobacteria. Viruses can cause declines in algal populations under natural environments.

Soil Formation and Quality

Although soil algae may be overlooked in many soil ecosystems, they make significant contributions as geologic agents of soil formation. In addition they promote the maintenance of soil structure and improve soil quality.

Geological agents:

Algal activity may contribute to geologic weathering. Algae can inhabit the surfaces of rocks and, to some extent, the interior of porous rocks. They can form and enlarge the cavities they occupy. The depth to which the algae are found in rock is limited by light penetration, as is the case in soil environment. The mechanism by which algae penetrate rock is not completely understood; however, several algal species are capable of secreting acidic substances (e.g., carbonic acid from respiratory carbon dioxide) which may contribute to the weathering process. Soil algae also promote the release of nutrients from insoluble compounds.

The soil algae are important in stabilizing and improving the physical properties of soil. They participate in the formation of soil organic matter through both the exudation of organic compounds and, upon their death, the contribution of their cellular material to soil organic matter. The latter process can be very important in desert ecosystems. Organic matter can help reduce erosion, facilitate water infiltration and root development, and, in agroecosystem, facilitate tillage. The soil algae produce extracellular polysaccharides that stabilize soil aggregates, thus promoting the establishment of higher plants. In particular, the extracellular polysaccharides assist in binding colloidal clay or humic particles through adsorption, adhesion or cation bridging.

Microbiotic crusts:

Soil algae can form microbiotic crusts or mats, which are often mistaken for mosses or lichens. These surface crusts can retard erosion and reduce the loss of water by evaporation, thus increasing the storage of water. They may also influence nutrient cycling and serve as indicators of pollution. These crusts develop between open shrub and grass communities of arid and semiarid environments and are distinguishable from crusts formed by chemical or physical processes. Cyanobacteria and algae are the major components of many microbiotic crusts, although mosses, lichens, liverworts, fungi, and associated microfauna may also be found. Microbiotic crusts are easily damaged by trampling animals and vehicular travel.

Soil Quality:

Soil quality (or soil health) is a critical element in sustainable agriculture. However, soil quality is difficult to define and even more difficult to evaluate. One possible way to evaluate soil quality is to use soil algae as indicators. Because of their cell structure, nutrient requirements, biochemical similarity to higher plants, and rapid growth rates, algae may have value as indicator organisms for anticipating crop responses to fertilizers and pesticides.

All algal activity is not beneficial to soil quality. Algal growths can seal the soil surface, reducing water infiltration and soil moisture. Algal crusts may also create a barrier to gas exchange with the atmosphere and inhibit seedling emergence. Conspicuous surface growths of algae on sports turf and ornamental grasses also cause aesthetic problems. In managed turf, algal problems are often caused by unsatisfactory drainage or irrigation practices, but other factors such as fertilizer treatment may be involved.

Biofertilizer:

Soil algae, especially the cyanobacteria with their ability to fix dinitrogen, have the potential to be biofertilizers; however, this application is not widespread. Cyanobacteria have been used to reclaim saline soils in India and as a source of fertilizer nitrogen in rice production, especially in China and India. Cyanobacteria have contributed to as much as 50 kg N ha^{-1} in tropical rice paddy agroecosystems. The ability of the inoculum to become established is a primary factor limiting the use of this technology.

Figure 1: Some representative soil cyanobacteria(a-d and f) and eukaryotic alga (e).

Figure 1: Diagram of a cross section of a cell of cyanobacterium: Cm= cytoplasmic membrane, G= Gas vesicles, Gl= glycogen granules, Iv= intralamellar vesicle, L= lamelle, N= Nucleus, Pb= Polyphosphate body, Ph= polyhedral body, Py= phycobilisomes, R= ribosomes, S= sheath, Sg= sytructured (cyanophycin) granule, W= wall