

Soil & Water Microbiology

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(Practical Part)

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What is a *soil*?

Soil is the top layer of the Earth's lithosphere, formed from weathered rock that has been transformed by living organisms.

Soil formation factors

The process of soil formation that starts from the host rock, soils' base component, may follow a different course depending on the following soil formation factors:

- climate**
- water**
- living organisms**
- surface configuration**
- human activity and**
- time (soil's age)**

We Study Soil Because It's A(n)



TYPES OF SOILS

- **Eight major types**
 - **Alluvial soils**
 - **Black soils**
 - **Red soils**
 - **Laterite soils**
 - **Desert soils**
 - **Mountain soils**
 - **Saline and Alkaline soils**
 - **Peaty and Marshy soils**

ALLUVIAL SOIL

- Formed by deposition of alluvium by rivers.**
- Contribute greatly in agricultural development.**
- Sandy, pale brown composition.**
- Found in lower areas.**
- Flooded every year.**

ALLUVIAL SOIL

- **Characteristics:**
 - **Transported soils.**
 - **Coarser in upper section and finest in delta.**
 - **Light to dark in colour.**
 - **Rich in Potash & Humus.**
 - **Poor in Phosphorous and Nitrogen.**
 - **Highly fertile, good for all crops**

BLACK SOIL

- Also known as Black Cotton soil.**
- Dark grey to Black in colour.**
- High clay content.**
- Highly moist retentive.**
- Highly suitable for cotton.**
- Rich in iron, lime, calcium, Magnesium, carbonates, and alumina.**
- Poor in Phosphorous, Nitrogen and Organic matter.**

RED SOIL

- **Formed due to weathering of old crystalline rocks.**
 - **More sandy and less clayey.**
 - **Rich in iron, small amount of Humus.**
 - **Poor in phosphorus, nitrogen and lime.**
 - **Slightly acidic and do not retain moisture.**
 - **Porous and Friable.**

LATERITE SOIL

- **Latin word meaning brick.**
 - **Formed under high temperature and rainfall with wet and dry spell.**
 - **Silica is leached due to high rainfall.**
 - **Remnants of iron and aluminum oxides left behind is know as Laterite.**
 - **Brown to Yellowish colour.**
 - **Becomes hard when exposed to atmosphere.**
 - **Used as building material.**
 - **Rich in Iron.**
 - **Poor in Lime, Potash, & Magnesium.**

DESERT SOIL

- Contains soluble salts.**
- Originated by Mechanical disintegration & wind deposit.**
- Porous and coarse.**
- 90% sand & 5% clay.**
- Rich in Nitrates & Phosphates.**
- Poor in Nitrogen & Humus.**
- Friable, sandy & low moist content.**

MOUNTAIN SOIL

- Found in hill slopes.**
- Formed by deposition of organic matter from forest.**
- Rich in humus.**
- Poor in Potash and Lime.**

SALINE & ALKALINE SOIL

- Contains salts like Sodium, Magnesium, Calcium.**
- Infertile, unfit for cultivation.**
- Sandy to loamy in texture.**

PEATY AND MARSHY SOIL

- Occur in Humid region.**
- Formed by accumulation of organic matter.**
- Black in colour.**
- Highly acidic and heavy.**

Soil functions

- **It takes part in primary biomass production and it allows anchoring for plants, supplying them with water as well as the essential mineral products.**
- **The decomposition processes of the organic matter and the accumulation of humus take place.**
- **Due to its chemical composition and physical properties soil forms a habitat for massive amounts of microorganisms and other living organisms.**
- **Within this habitat soil serves various filtration and buffering functions which protect the ecosystems against the excess flow of unwanted substances from other biosphere elements.**

Soil's composition (Types of the soils)

Soil is composed of **mineral** and **organic** solid particles, air, soil solution, and living organisms which occur in this - edaphon. The proportions of particular components within soil stay more or less at the same level for the given kind of soil (Fig. 1.1).

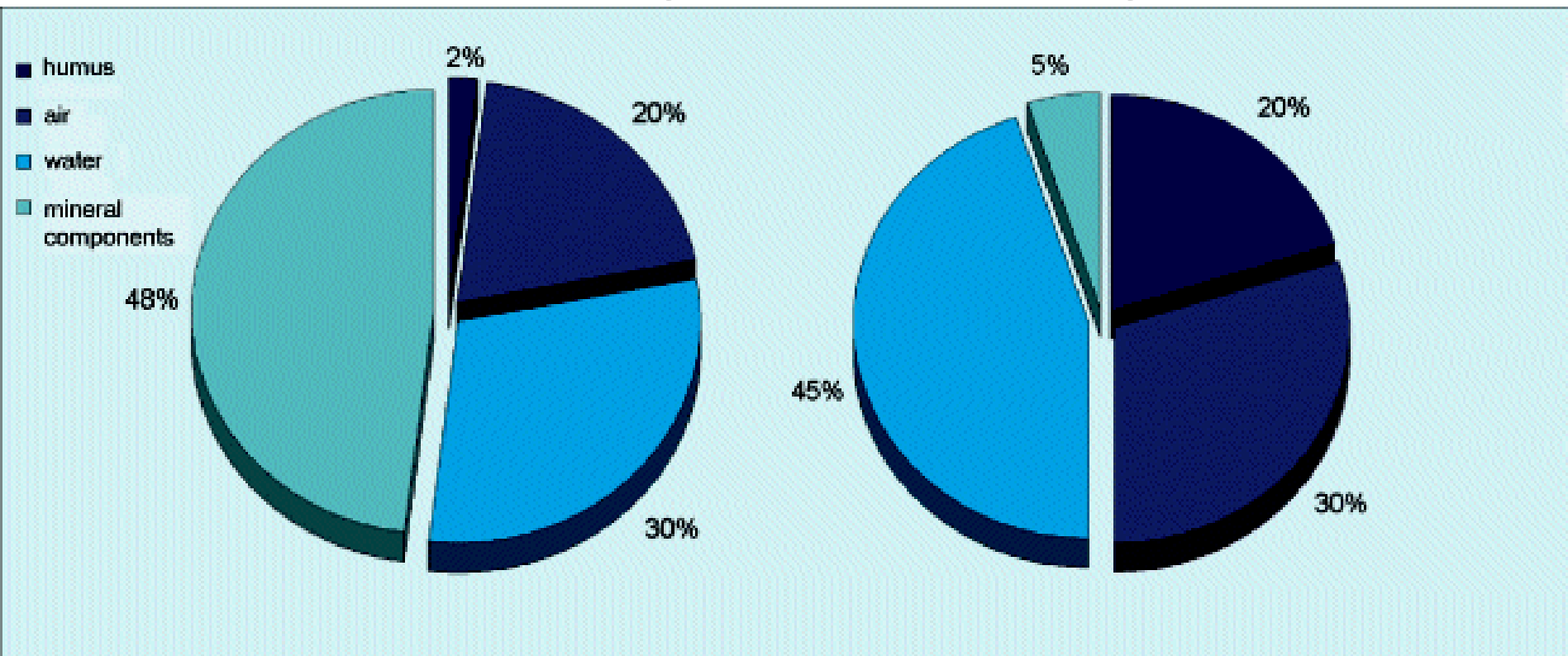


Fig. 1.1. Average fraction of particular phases of the soil:
mineral (on the left) and **organic** (on the right).

Mineral Compounds

- **They occur in soil in a form of particles of various sizes.**
- **The smallest fraction consists of mineral colloids built from the aluminosilicates, hydrated silica, aluminium and iron hydroxide.**
- **Soil colloids strongly absorb oxygen, water and crucial nutrients, while they also create habitat for microorganisms.**

Organic substances

- Soil's organic substances are created by a residue of dead plants, animals and microorganisms, which are decomposed by the soil-inhabiting microorganisms.
- Decomposition of the organic substances consists of different microbiological and physical-chemical processes called **humification** and its end-products are humic substances (**humus**) which are partially in a colloidal state.
- The organic colloids are a source of food for the microorganisms. Moreover, in the connection with silty particles, they give soil an adequate structure. Humus favours the growth of higher plants due to the ability to absorb water as well as the adsorption and exchange of the mineral compounds.

Soil solution

The soil solution consists of water with dissolved organic and mineral substances as well as gases. The water is held in soil due to the capillary forces acting within its aggregates.

The chemical composition of the soil solution constantly changes, depending on, among other things, the temperature fluctuations and the amount of water which either dilutes or concentrates the soil's solution. Nevertheless the microorganisms that live there have constant access to the ammonium, phosphate and potassium salts as well as the nitrates.

Moreover, easily available organic compounds such as monosaccharides and amino acids are found in the soil solution. Soil water provides favourable conditions for various organisms (not only for microorganisms but also for plants):

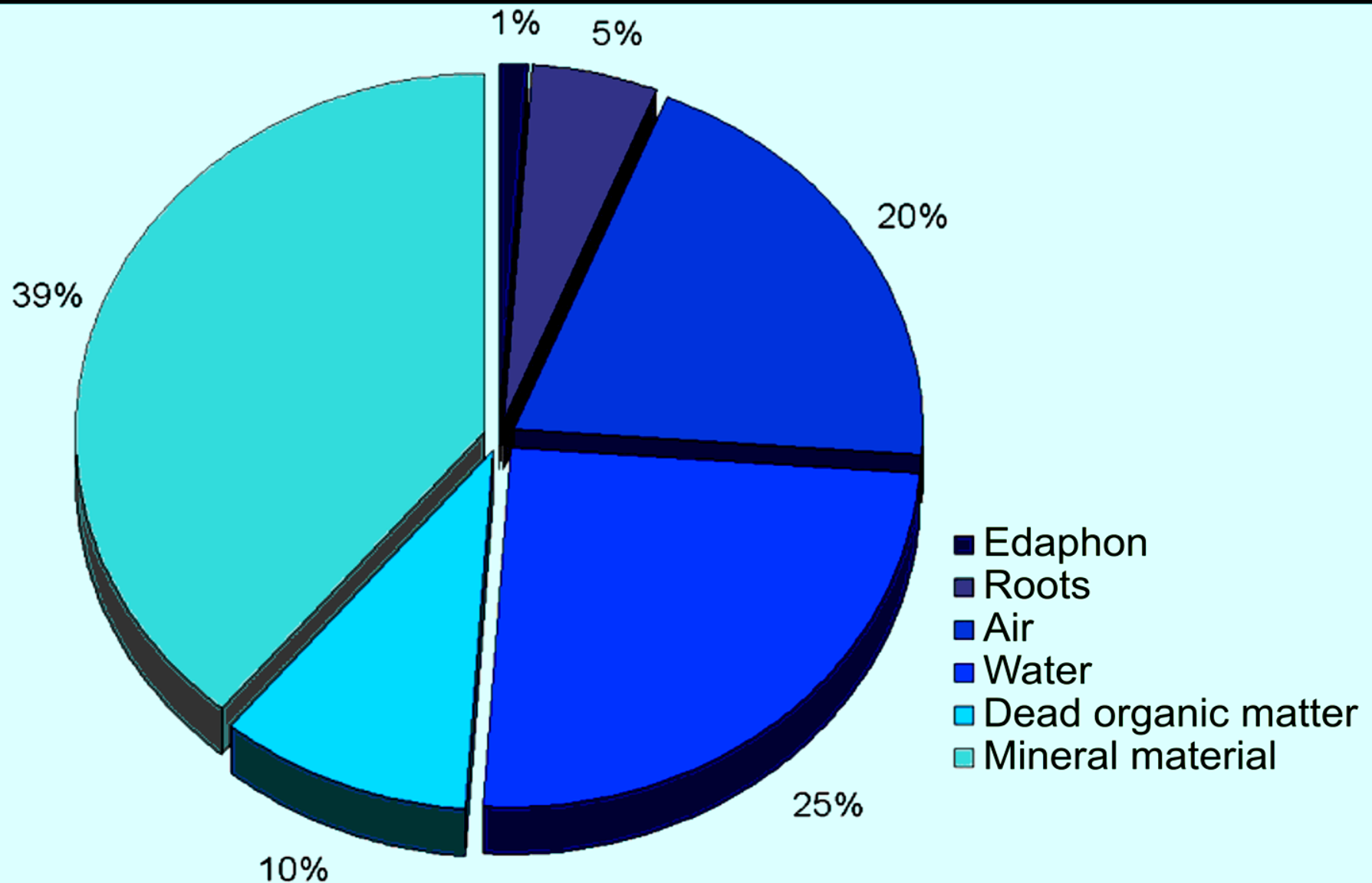
- It transports building and energy substances along the capillaries,**
- It influences the aeration, the amount and the quality of nutrients, the osmotic pressure and the pH of the soil solution.**

Soil atmosphere

- Soil atmosphere is the air in soil that fills out water-free spaces between the solid particles. Moreover the air saturates the soil colloids.
- The amount of air in soil varies between 8-35% of the soil's volume. Gases that constantly occur in the air are: N_2 , O_2 , and CO_2 . The transient gases are: NH_3 , H_2 , CO , NO_x , SO_2 , H_2S , CH_4 , C_2H_6 as well as other volatile organic substances (butyric acid, alcohol, esters).
- Soil air is usually saturated with water vapor and contains 10 times more CO_2 than air in the atmosphere.
- The change from the oxygen to oxygen-free metabolism (the reduction of sulfate, denitrification) occurs in soil when the concentration of O_2 falls below 1%. As a result, we can observe the growth of the anaerobic microorganisms.

Edaphon

- The organisms living in soil create a community called the *edaphon*. These are *bacteria*, *fungi*, unicellular *algae*, vascular plants and animals especially invertebrates that occur in the surface layer of soil.
- Due to the variety of their metabolic abilities the soil microorganisms ensure the permanence (continuity) of element cycles in nature. The effect of their activities is not only the mineralization of organic compounds but also the changes of mineral compounds, which have a big impact upon the development of the green plants.
- *Edaphon* constitutes about 1-10% of the dry mass of the soil organic matter (Fig. 1.2).



**Fig. 1.2. Percentage volume of soil components.
One % of soil is occupied by edaphon**

Horizons

- Soil is composed from layers are called *Horizons*.
- Each *horizon* is identified with the letters from the top **O**, **A**, **E**, **B** or **C**. not every soil has the all horizons.

-O horizon is formed from plant and animal.

-A horizon is the 1st mineral horizon and less organic.

-E horizon is similar to **A horizon** but less darkness and lightly colored.

-B horizon is the zone of **illuviation** because nutrients and minerals are deposited or accumulated.

-C horizon is unconsolidated parent material.

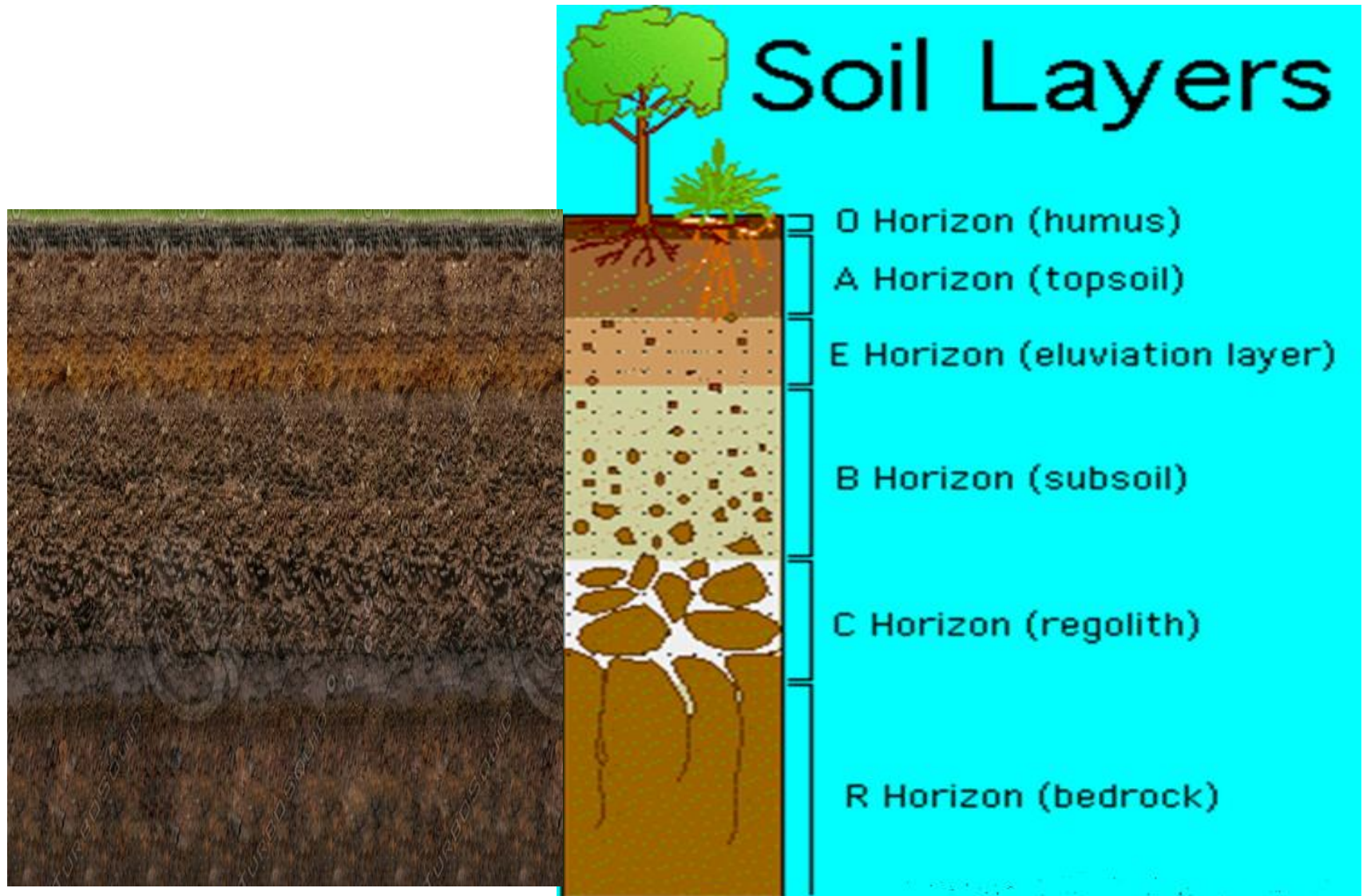


Fig. 1.3. Soil *Horizons*

I- Soil Physical Characters

1- Soil Texture

Soil Texture is measurement of the proportion of mineral particles of different sizes that are found in the same sample of soil (sand, silt, clay).

Soil Textural Classes:

The combined portions of sand, silt, and clay in a soil determine its textural classification.

- 1- **Sand** particles range in size from **0.05–2.0 mm**.
- 2- **Silt** ranges from **0.002–0.05 mm**.
- 3- **Clay** fraction is made up of particles **less than 0.002 mm** in diameter.

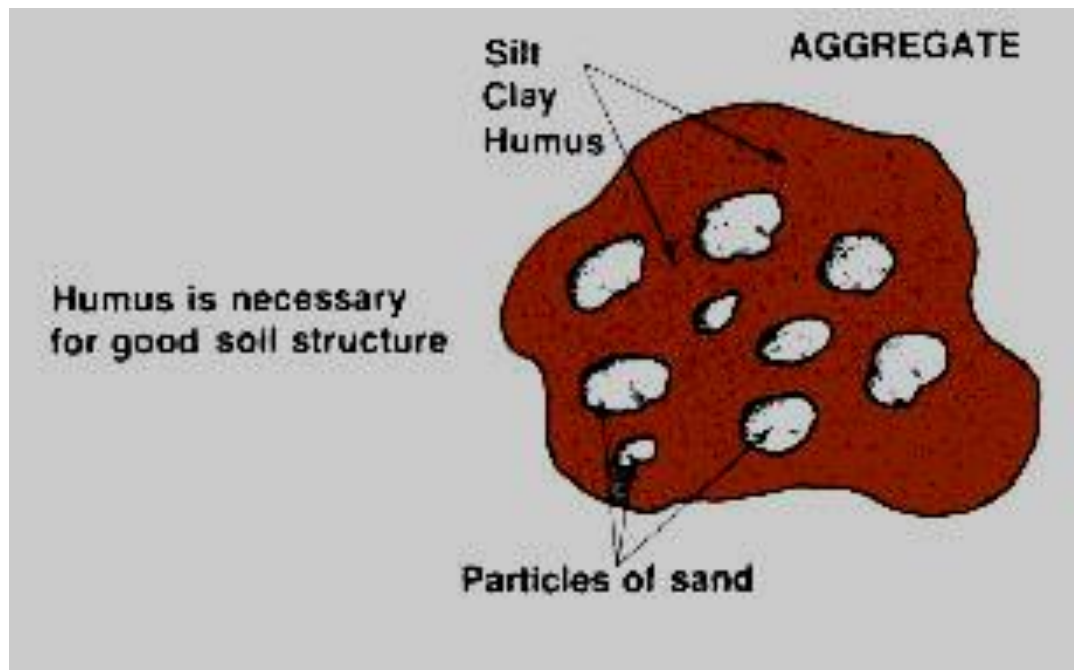
***Once the sand, silt, and clay percentages of a soil are known, the textural class can be read from the **textural triangle**.**

Effects of Soil Texture:

- Soils are more **cohesive** when they have more **fine particles** (Clays).
- Soils are more **loose** when they have more **coarse particles** (Sand).
- Different combinations of coarse and fine contents produce different soil textures.
- A **loam** is a mixture of sand, silt and clay: sandy clay loam is best in landscapes.
- Many other inclusions, such as cobbles, boulders.

2- Soil Structure

Soil structure is defined by the way individual particles of sand, silt, and clay are assembled. Single particles when assembled appear as larger particles. These are called **aggregates**.



3- Soil Pores

- **Pores** are spaces not occupied by soil solids (mineral or organic).
- **Matrix pores**: spaces between particles
- **Non-matrix pores**: outside of aggregates by worms, root channels, cracks, etc.

Classes:

Macropores are between **30-100 μm** diameter.

Micropores are smaller than this.

4- Soil Consistency

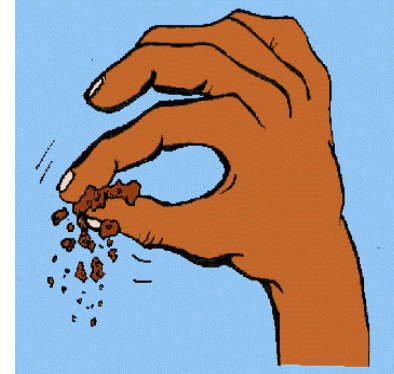
- Describes the general organization of the soil.
- Hold a moist sample between the thumb and forefinger, and gently squeeze it until it falls apart.
- The soil is classified by the following categories;
 - **Loose:** You have trouble picking out a single sample and the structure falls apart before you handle it.
 - **Friable:** The sample breaks with a small amount of pressure.
 - **Firm:** The sample breaks when you apply a good amount of pressure and dents your fingers before it breaks.
 - **Extremely Firm:** The sample can't be crushed with your fingers (you need a hammer!).

Soil Consistency

Loose*



Friable



* Soils with “single grained” structure always have loose consistence.

Firm



Extremely Firm



II- Soil Chemical Characters

1- Soil pH

- The course of microbiological processes in soil, largely depends on the reaction pH, since this factor determines enzymes' activity and the process of transportation.
- Soil's pH influences the solubility and nutrient availability. **Iron** and **manganese** are available only under the conditions when the pH level is **low**, whereas **molybdenum** is available only in **high** pH.
- The pH value of soil depends on its chemical make up, however during the biological processes of decomposition of organic matter, changes in pH may occur as a result of metabolism and the microorganisms' physiology.

- The acidity of soil can increase as the result of acid rains, fertilization, or settling by the sulfur oxidizing bacteria etc., and this influences a range of metabolic transformations.
- One of the most sensitive reactions to soil's pH is nitrification. That is the transformation of NH_4^+ to NO_3^- . These ions also significantly affect soil's pH. The absorption of ammonium ions (NH_4^+) by microorganisms from the environment contributes to soil's acidification, whereas the assimilation of nitrates (NO_3^-) to its alkalinity. These changes then affect the solubility of other salts and their availability to microorganisms.
- Many of the known species of bacteria can grow in pH 4-9. However the optimal pH conditions for bacterial growth is at the pH level of 6.5-8.0.

- Many acid tolerant microorganisms may grow in a range of **pH 1-6**, the extreme acidophiles successfully grow in pH values of 1-3. Among them are some species of *Thiobacillus*, *Thermophilus* and *Sulfolobus* that oxidize mineral sulfides to form sulfuric acids.
- Most of the fungi prefer acidic reaction of the environment. Fungi, as a group are extremely acidophilic (the optimal growth conditions are between the pH 4-6).
- The bacteria that belong to a group of *Nitrosomonas* are moderate alkaliphilous microorganisms that best grow in the pH level between 7.3-9.6.
- Soil's solution is characterized by buffer properties which give the ability to withstand reaction changes to a certain extent.

2- Soil *Anion & Cation* Exchange Capacity

- Soils can possess both positive and negative charge.
- The ability of positively charged materials in soil to hold negative ions (H_2PO_4^-) is called *Anion Exchange Capacity* (AEC).
- The ability of negatively charged materials in soil to hold positive ions (K^+ , Ca^{+2}) is called *Cation Exchange Capacity* (CEC).

3- Toxic compounds

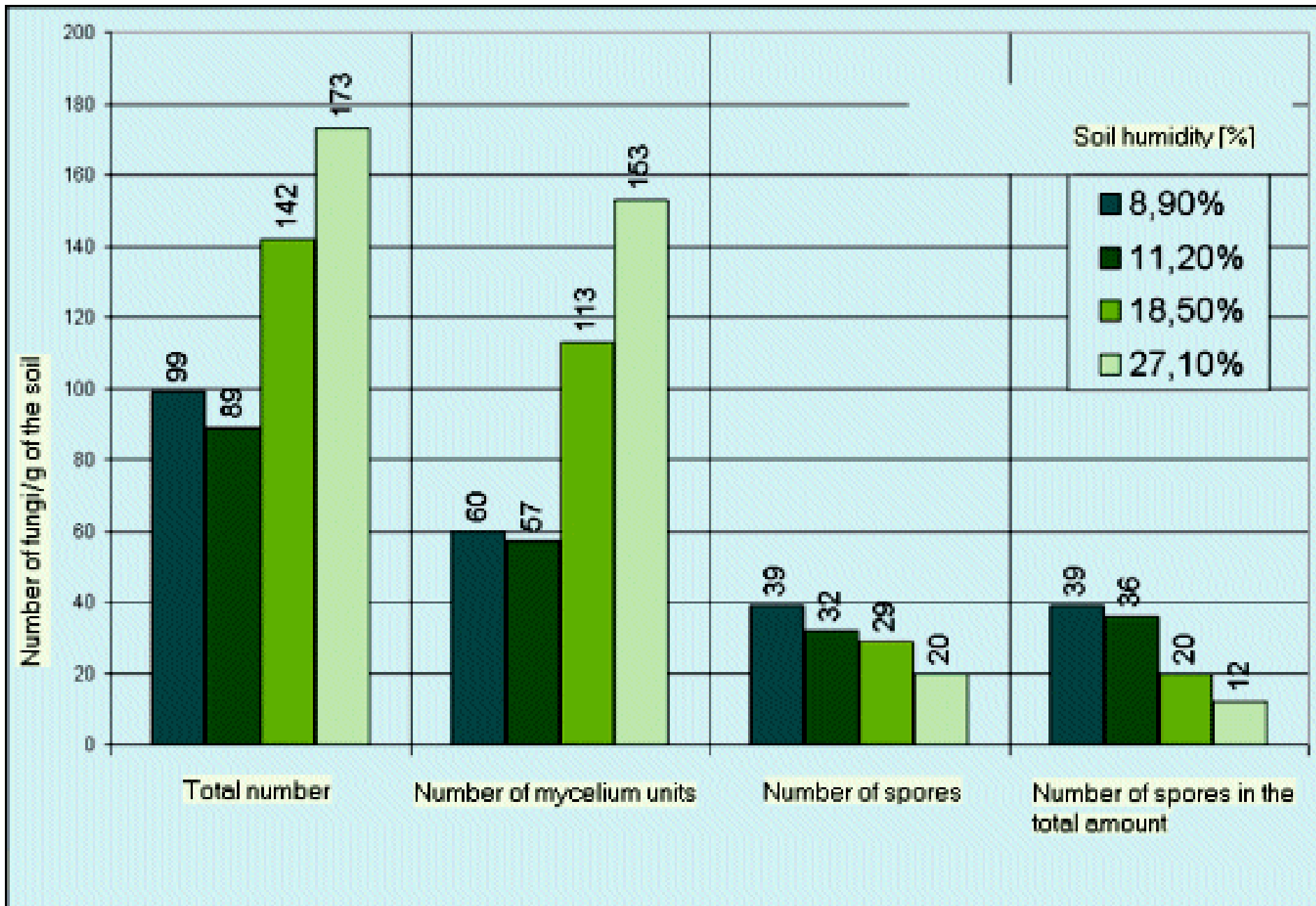
- **The presence of toxic compounds can delay or completely stop microbiological processes in soil.**
- **Particularly toxic compounds are pesticides, aliphatic and aromatic hydrocarbons, formaldehydes, chloroorganic compounds, heavy metals and salts occurring in high concentrations.**

III- Soil Abiotic Factors

1- Water

- All microorganisms require water-containing environments for proper development.
- Water allows microorganisms' migration within soil, diffusion of substrates and nutrients into the inside of the cell as well as the elimination of metabolism's products.
- Also too high concentration of nutrients as well as excessive hydration slows down or stops all together the microorganisms' development.
- Water strongly bonded with solid particles of soil may not be available for the microorganisms.

- An excess of water in soil lowers the diffusion of oxygen and nitrogen as well as favors the development of predators that feed upon the bacteria. Insufficient amounts of water may prevent the predatory protozoans from relocating and as a result it supports the development of the bacteria.
- In general moulds and yeast demonstrate much higher tolerance to environmental dehydration than bacteria do.
- The aerobic degradation of organic compounds in soil is most efficient in soil humidity between 50-70% of its maximum water capacity.



Relation between humidity of the soil and number of Fungi

2- Osmotic pressure

- The development of microorganisms is influenced to a large extent by the osmotic pressure that is connected to the humidity of soil that gradually increases as the soil dries up.
- In soils of medium humidity the solution pressure fluctuates between 0.5-5 atm.
- In salty soils it may reach 100 atm. Inside the microorganisms' cells it varies between 3-6 atm.
- The osmotic pressure that is higher in soil solution than inside the cells interferes with the process of water absorption by the microorganisms' cells and subsequently slows down their growth.

3- The Redox potential

- **The Redox potential** is the measure of the tendency of a compound to accept or donate electrons.
- In soil solutions salts of elements with variable valence are dissolved. These for instance are: Fe, Mn, S. The oxygen and oxygen-free processes depend on the relationship between the oxidized and reduced compounds of $\text{Fe}^{3+}/\text{Fe}^{2+}$, $\text{MnO}_2/\text{Mn}^{2+}$, $\text{So}_4^{2-}/\text{S}^{2-}$ and on the level of oxygen within soil.

- **Over-dried soil and the consequent better aeration increases the mineralization and oxidation processes.**
- **In contrast, an excess in the soil's humidity causes the elimination of oxygen, which is being controlled by microaerophiles and anaerobes; they reduce the soil redox potentials, which stimulates the reduction and fermentation processes.**

4- Temperature

Soil microorganisms differ because of their thermal tolerance and optimal growth temperatures. Considering microorganisms' sensitivity to temperature the following may be singled out:

- Psychrophilic**
- Mesophilic**
- Thermophilic**

- For the *psychrophilic* the growth temperature ranges between -5 to +25°C, for the *mesophilic* it is 15 - 45°C and for *thermophilic* it ranges from 40 - 70°C.
- Despite the fact that some species may have a bigger or smaller tolerance to temperature changes, most belong to the *mesophilic* group that tolerates temperature of about 30°C.
- The organisms that grow in low temperatures of about 0°C contain in their cell membrane special lipids that maintain its semi-fluidity. **Thermophilous** types have lipids with high melting points.

- **Excess increase of temperature causes a serious decrease in the biosynthesis process due to the greater use of energy for respiration, decrease in production output, and the appearance of side effects. In temperatures below 6°C microorganisms limit the life processes, and go into the state of *anabiosis* or into the resting forms.**
- **In soils the temperature may reach 70°C at its surface at noon and demonstrate daily fluctuations of about 50°C. The changes in temperature on the surface during a 24h period do not have any influence on the temperatures in deeper levels of the soil profile.**

5- Oxidation

- Oxygen is among the crucial factors that control the growth conditions of microorganisms, it affects the ability or lack of growth, it influences the speed of growth, mass increase and the cell physiology thereby affecting, productivity and the speed of particular metabolite production.**
- The microorganisms are grouped into many categories depending on their Oxygen requirements.**

Table 6.4 Oxygen relationships of microorganisms

| Group | Relationship to O ₂ | Type of metabolism | Example ^a | Habitat ^b |
|------------------|--|--|--|--|
| Aerobes | | | | |
| Obligate | Required | Aerobic respiration | <i>Micrococcus luteus</i> (B) | Skin, dust |
| Facultative | Not required, but growth better with O ₂ | Aerobic respiration, anaerobic respiration, fermentation | <i>Escherichia coli</i> (B) | Mammalian large intestine |
| Microaerophilic | Required but at levels lower than atmospheric | Aerobic respiration | <i>Spirillum volutans</i> (B) | Lake water |
| Anaerobes | | | | |
| Aerotolerant | Not required, and growth no better when O ₂ present | Fermentation | <i>Streptococcus pyogenes</i> (B) | Upper respiratory tract |
| Obligate | Harmful or lethal | Fermentation or anaerobic respiration | <i>Methanobacterium formicicum</i> (A) | Sewage sludge digestors, anoxic lake sediments |

^a Letters in parentheses indicate phylogenetic status (B, *Bacteria*; A, *Archaea*). Representatives of either domain of prokaryotes are known in each category. Most eukaryotes are obligate aerobes, but facultative aerobes (for example, yeast) and obligate anaerobes (for example, certain protozoa and fungi) are known.

^b Listed are typical habitats of the example organism.

- Pores that occur in typical soils are 50% water and air.
- The biodegradation process occurs quickest when the content of oxygen in soil's air is higher than 0.2 mg O₂/l.
- 70% of the oxygen contained in soil is used by microorganisms, 30% by plants' roots, whereas the chemical processes use up only trace values of oxygen.
- Oxygen-free conditions occur in soils where the oxygen content is lower than 1%.

- In the cases when oxygen supply is not possible, the biological organic matter decomposition process is conducted by the anaerobic bacteria. They utilize an oxygen source in compounds such as **sulfate** or **nitrate**. In oxygen-free conditions the organic matter decomposition processes are slowed down and are less energetically effective.

6- The content of nutrients

- In order to build up microorganisms' biomass, besides carbon, other nutrient such as the following are essential: nitrogen, phosphorus, sulfur, calcium, magnesium, potassium. Particularly important elements are nitrogen and phosphorus which are essential in the production of proteins and nucleic acids.
- Fertile soils contain all the essential components at adequate proportions whereas in contaminated soils the proportions between the particular elements are disturbed. It is believed that the weight ratio of carbon to nitrogen and phosphorus in soils should be at about 10:1:0.1.
- Calcium improves the soil's physical and chemical qualities as well as its structure.

7- Light

- **Light penetrates only the top few cm of soil.**
- **The quantity of illumination depends on the type and density of plants growing upon it.**
- **Light is only essential for algae that carry out the process of photosynthesis.**
- **Light exposure affects earthworms' activity since they move up to the surface at night in search of food and in order to reproduce.**