INTRODUCTION TO ENVIRONMENTAL MICROBIOLOGY

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The publication was prepared based on delivered materials

ISBN 83-7085-880-5

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The INTRODUCTION TO ENVIRONMENTAL MICROBIOLOGY has been developed by academic teachers from Wroclaw University of Technology, Poland in the frame of international project Socrates Minerva CELL TALK–88091–CP-BE-2000-Minerva-ODL realized together with partners from Belgium, Ireland, Bulgaria, Portugal and Netherlands. The project was coordinated by prof. Chris van Keer from Katholieke Hogeschool Sint Lieven in Gent, Belgium. The book is addressed to students of environmental engineering, biology, biotechnology, biochemistry and to students of other specializations interested in increasing their knowledge about microorganisms living in environment and in solving environmental problems with the use of microorganisms capable of degrading xenobiotics.

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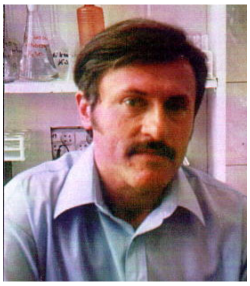
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Acknowledgements

The authors express their gratitude to M.Sc. Maria Pawlik, M.Sc. Agnieszka Trusz, Barbara Umińska, M.Sc. Henryk Małysa, dr Aleksandra Obara and dr Marta Chyla for technical assistance and research cooperation.

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1. Microbiology of soil

Contents

Aims

After studying this chapter you should know what kind of microorganisms exists in soil, what physical, chemical and biological factors influence their development. You should be familiar with the roles microbes play in soil development and which forms of interactions we can observe in soil biocenosis. Most importantly, this chapter will provide you both a full data in understanding the role of microorganisms in the soil environment and a basic information about the known possibilities of soil remediation.

Orientation

In this chapter we have characterised the soil environment and discussed the soil microorganisms in combination with factors influencing their activity and development. Also, the relations between organisms living in soil are presented.

Prior knowledge

Do you know what viruses, bacteria, fungi, algae and protozoa are? What is their morphology? What can you say about metabolism in microrganisms? In first instance you have to take care to know the catabolic reactions performed by the microorganisms.

Study advice

First you have to read the whole chapter. Then look at the glossary at the end and try to explain all unknown words. If you have any problems with understanding of the presented material you should return to the chapters describing the cell structure and metabolism.

1.1. Soil

What is 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)

Soil functions

Soil is a complex formation that allows the functioning of soils' ecosystems.
- It takes part in primary biomass production and it allows anchoring for plants, supplying them with water as well as the essential mineral products.
- There 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

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).

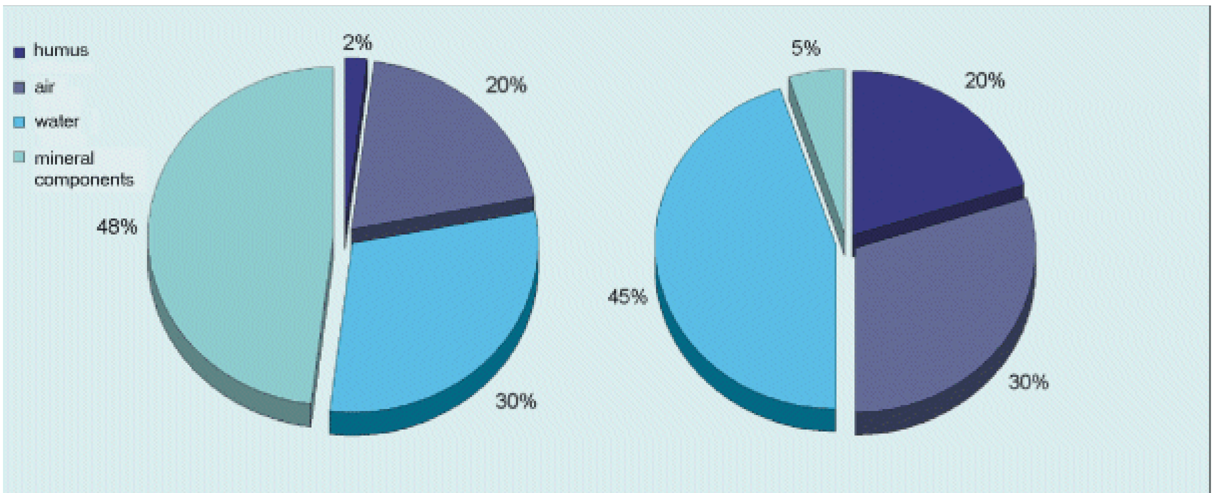

Fig. 1.1. Average fraction of particular phases of the soil:
mineral (on the left) and organic (on the right)

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. The colloids are a soil component that determines the water-air relationship.

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).

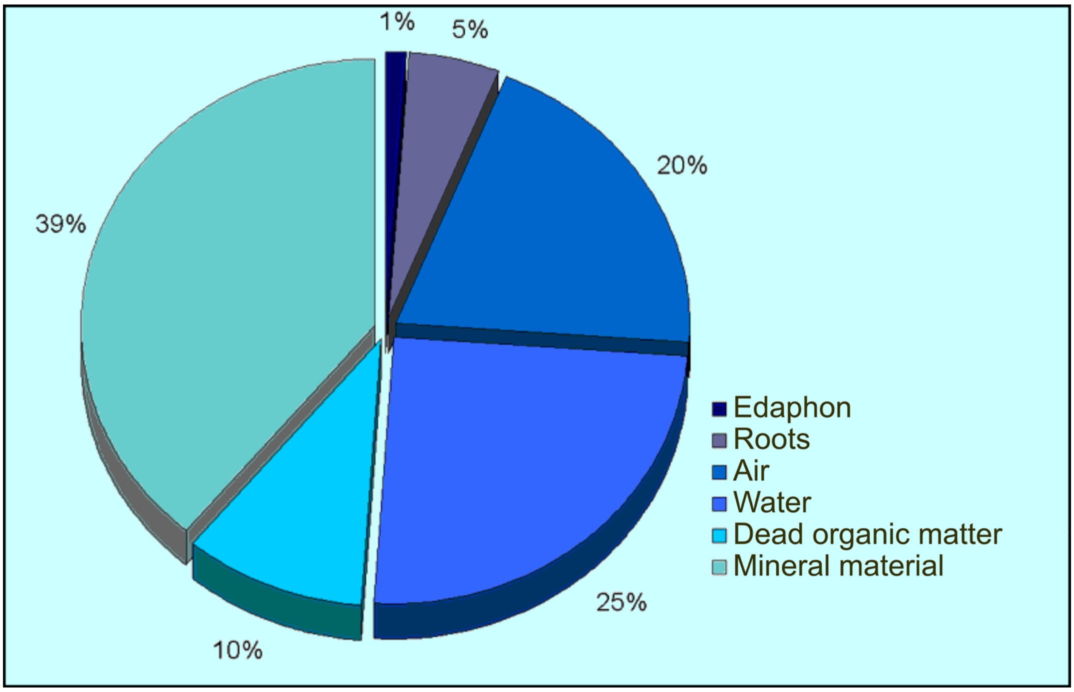

Fig. 1.2. Percentage volume of soil components.
One % of soil is occupied by edaphon

1.2. Edaphon

Based on their size, the organisms living in soil may be categorized into three groups:
Microbiota (not visible with the naked eye)
viruses, bacteria, fungi, protozoa, algae
Mesobiota (0.2-2 mm)
nematodes (eelworms), mites, myriapods, wingless insects, snails, and some small plants
Macrobiota (>2 mm)
earthworms, larger insects, moles, rodents such as field mice, and roots of large plants.

1.2.1. The characteristics of soil microorganisms

Viruses

- Viruses lead a strictly parasite existence - they reproduce within bacteria, plants, animals and human cells.
- The most important kind of viruses in the soil environment are the viruses living in bacteria cells, called bacteriophages (phages).
- The role of phages in the soil environment depends on their ability to eliminate some populations of bacteria and on selecting the microorganisms both in a negative and positive way. The example of their negative influence are the phages that attack the root nodule bacteria (*Rhizobium*) which are the cause of the decline of papilionaceous plants crops.

Bacteria

- Bacteria constitute the basic mass of all soil microorganisms. They are characterized by high metabolic activity.
- Most soil bacteria are characterized by the ability to adhere to surfaces of the mineral molecules and to the soil colloids.
- Especially high numbers of bacteria gather around the residue of plants' and animals' tissues as well as in animal dropping that finds its way into the soil. The environment that is especially suitable for the development of the bacteria are the plants' roots and their other underground parts.
- Soil bacteria can be subdivided into two groups: those that always occur in each one of the soils' type (autochthonous) and the ones that grow only after high amount of the organic matter discharge into the soil (zymogenous).
- The largest group of soil bacteria is represented by the actinomycetes and rod-coccus bacteria that belong to the *Arthrobacter* genus.

Actinomycetes

The Actinomycetes are (chemo) organotrophic bacteria. They form elongated branched out mycelium-like threads that contain a large number of prokaryotic cells. The width of the threads is 1-5 μm. They mainly live in soil or upon decomposing plants. Most of them lead a saprophytic type of life, and some are pathogenic to plants and animals (for example: *Streptomyces somaliensis*, *Actinomyces israelii* and *Nocardia asteroides* cause the subcutaneous infections of feet called mycetoma).
Their growth abilities in temperatures of 40-50 ^{o}C give them a wide range of decomposition potential of various substances. The Actinomycetes degrade steroids, lignin, chitin, hydrocarbons, fatty and humic acids, which are not easily decomposed by other bacteria. During the decomposition of the above they produce aromatic compounds. The characteristic smell of freshly ploughed soil, especially in spring, comes from the actinomycetes bacteria. The smell is caused by the substance called geosmin (*1,10-dimetylo-9-dekalol)*, which is produced by *Streptomyces griseus.*
They are the aerobic bacteria, whereas a small group has the ability to conduct the metabolic processes in anaerobic conditions (*Actinomyces, Micromonospora).*
Many types of actinomycetes produce antibiotics such as erythromycin, neomycin,

streptomycin, tetracycline plus others as the by-product of metabolism.
About 90% of all actinomycetes isolated from soil are *Streptomyces.*

Rod-coccus bacteria

Club-shaped bacteria that belong to the *Arthrobacter* genus are dominant in numbers representative of the autochthonous soil microflora. They make up 2-60% of the whole population of soil microflora and are characterized by the tendency to form branching and coccus forms. The bacteria are polymorphic. In new bacterial cultures the bacteria grow in a form of long irregular rods whereas in old cultures, they create coccus forms. They are characterized by a high resistance to the environmental factors during the vegetative stage. Also, they are capable of surviving in dry soil for a few months, whereas most of the other bacteria that do not produce resting spores die out.
The bacteria have the ability to utilize a wide spectrum of organic compounds as a food substrate. They conduct biodegradation of not easily accessible compounds and may utilize many metabolites of other microorganisms including various polymers, growth factors and the amino acids produced by microorganisms.
The bacteria which utilize the cellulose (*Cellulomonas)* also belong to the club-shaped forms.

Fungi

- Fungi belong to a group of eucaryotic organisms which are the absolute heterotrophs. Most of them belong to the group of aerobes or fermenting fungi. They take the carbon and energy to build their own cells from the decomposition of the organic compounds. Fungi do not have any chlorophyl. In contrast to bacteria the fungal cell wall contains chitin, glucans and other polysaccharides.
- They occur mostly in the upper layers of soil however they can be found as deep as 1 m.
- They get into symbiotic relationships with algae, insects and higher plants. Many species of fungi are pathogenic to humans, plants and animals.
- Their vegetative forms create thread-like shreds that are more or less branched out and usually multi cellular. Their thick weaves form mycelium or thallus. The individual cells are the size of about 10μm.
- The most common soil fungi are the genera of *Penicillium, Aspergillus, Trichoderma, Verticillium, Fusarium, Rhizopus, Mucor, Zygorhynchus, Chaetomium.*
- Fungi grow strongly in acidic soils and have crucial influence on changing of pH reaction.

The role of bacteria and fungi

- Both are the co-creators of soil's structure as they create humus - the most important component of soil that greatly influences its structure, sorption qualities and the richness in organic compounds.
- They have a great effect on the way of creation of crumb texture and a spongy structure of soil by producing mucous capsules, and like the filamentous bacteria and the fungi by their form of growth.

Soil phytoedaphon

Phytoedaphon consists mainly of algae and to a lesser extent the higher plants.
Algae are the main component of phytoedaphon. They are most numerous upon the surface of soil reaching deeper through ploughing, percolating water, animal activities and the ability to migrate. Two groups are distinguishable: algae colonies that live upon the surface-epiphytoedaphon and the ones that live in deeper layers - endophytoedaphon.

- Soil algae are obligatory photoautotrophs, however the ones living in deeper layers probably feed heterotrophically.

• They play a major role in soil's ecosystem and influence its qualities and stability. Through extracellular secretion they fertilize the soil and take part in nutrient discharge into the environment.
• Some blue-green algae (cyanobacteria) are capable of fixing the atmospheric nitrogen (*Nostoc, Anabaena, Scytonema, Tylypothrix).* Soil inhabited by these microorganisms contains 26-400 times more nitrogen. Due to the ability of nitrogen (N_2) and carbon dioxide (CO_2) assimilation they may be the first ones to colonize the nitrogen and organic carbon-free ground.
• About 2 thousand species of algae occur in soil. They are mainly:

- Blue-green algae - *Nostoc, Anabaena, Scytonema, Tolypothrix, Microcoleus, Schizothrix*
- Green algae - *Ankistrodesmus, Chlorella, Chlorococcum, Chlamydomonas, Characium, Klebsormidium*
- Diatoms - *Achnanthes, Cymbella, Eunotia, Fragilaria, Hantzschia, Navicula, Nitzschia, Pinnularia*
- Yellow-green algae - *Botrydiopsis, Heterothrix, Heterococcus, Pleurochloris*
- Euglenoids - *Euglena, Peranema*
- Red algae - *Porphyridium*

• Among the macrobiotic plant organisms that inhabit the soil environment higher plants dominate making up the basic element of biocenoses of all the land ecosystems.

Fauna of soil (Fig. 1.3-4)

The soil *microfauna* is represented by the protozoans, which mainly feed on bacteria. Their role is to conduct selection and rejuvenate the population of soil bacteria. Amoebae and flagellates dominate among them.

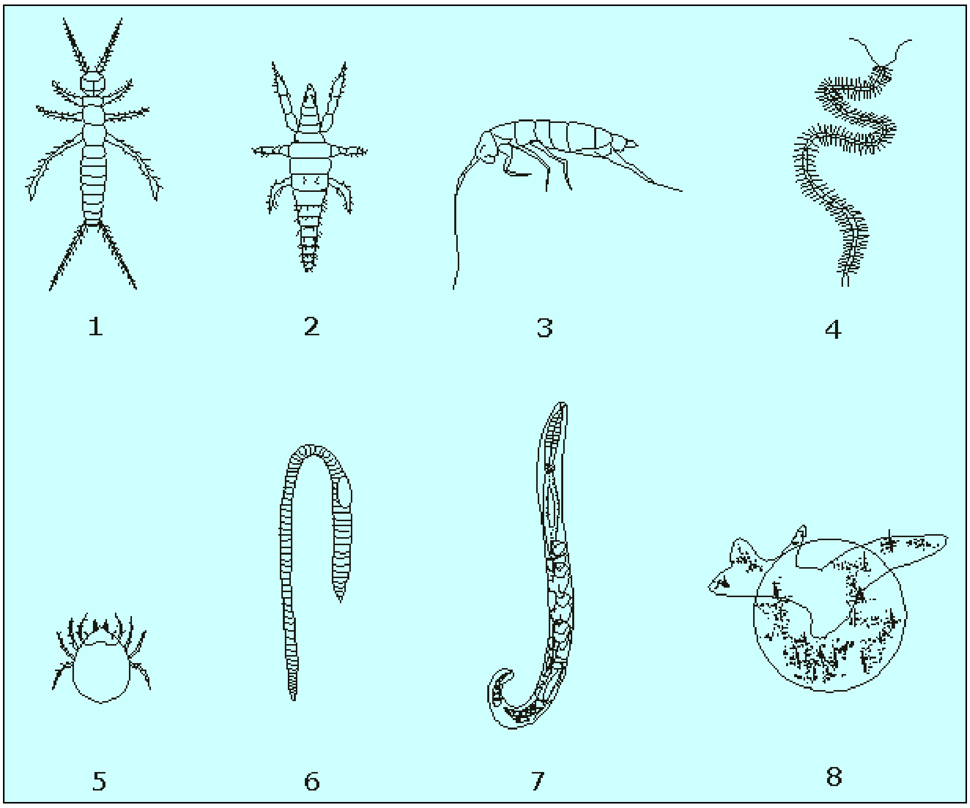

Fig. 1.3. The main types of soil fauna.
1 – Diplura (wingless insects), 2 – Protura (wingless insects),
3 – Collembola (springtails), 4 – Myriapoda (myriapods), 5 – Acarina (mites),
6 – Lumbricus terrestis (earthworm), 7 – Nematoda (eelworms), 8 – Rhizopoda (protozoans)

Mesofauna is represented by the nematodes (eelworms), snails, insects, myriapods, mites and other. They feed upon dead organic matter contributing to the formation of humus.

Macrofauna is represented by the earthworms, moles, rodents. The organisms break up soil material and carry it down to a significant depth. The earthworms play the most important role among the invertebrates, by feeding upon dead organic matter absorbing it along with the mineral part of the soil; non-digested residue mixed with mineral soil and the metabolites are excreted in the form of lumps (coprolites) what contributes to the formation of crumb texture of soil and to its loosening. During the period of one year, earthworms, on the area of one hectare, are able to pass 7 thousand kg of soil through their digestive tracts.

Fig. 1.4. The earthworm *(Lumbricus sp.)*

Due to animals' mobility and activities soil undergoes a constant mechanical mixing which in turn allows better aeration, oxygenation and water flow.

1.2.2. The number of soil microorganisms

The number and composition of soil microorganisms depends on the type of soil, its structure, humidity and on the content of the organic matter (Fig. 1.5-6).

Viruses
The exact number of viruses in soil is not known. Their mass is estimated at less than 0.01 tons/ha.

Bacteria

- The number of bacteria varies from a couple of million to a couple of billion cells per each 1g of soil. The highest number of bacteria occurs in a layer of cultivable soil at the depth of up to about 30 cm. In deeper layers their numbers quickly lower. In the cultivable layer of soil of about 30 cm thick there may be anywhere from several hundred kg up to a few tons of bacterial mass per each 1 hectare.
- In the vicinity of roots and upon their surface the bacteria find increased amounts of organic compounds such as organic acids, amino acids and vitamins that are excreted by plants. Therefore, in the layer around roots, called rhizosphere, the number of bacteria is several times higher than in soil far from the roots.
- In soils rich in organic compounds there live more bacteria, usually in 1 g of cultivable soil there may be between 0.5-5.0 billion bacteria (1.5-15 tons/ha).
- Acidic soils contain relatively low number of bacteria and a large number of fungi.

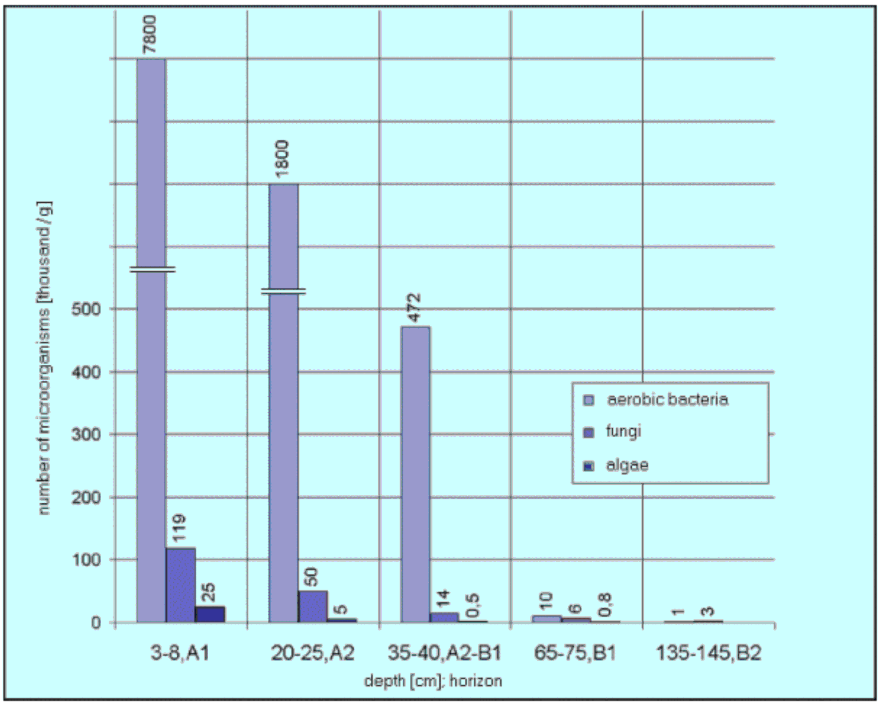

Fig. 1.5. Relation between the number of microorganisms in soil and depth

Fungi

- Fungi, excluding yeast, occur in the forms of mycelium or spores. The number of such units per 1 gram of soil may reach several tens of thousand, among that spores constitute anywhere from several to a few dozen percent depending on the soil's humidity and the organic substances available.
- Fungi are most widely represented in acidic peat and forest soils. There they may be more numerous than the bacteria.
- The main mass of fungi is found in the upper 20-30 cm layer. The combined mass of fungi in the upper layers is almost identical to that of bacteria and in forest soils it may even be greater. On average it is between 0.001-1.0 billion fungi (about 1.5 tons/ha).

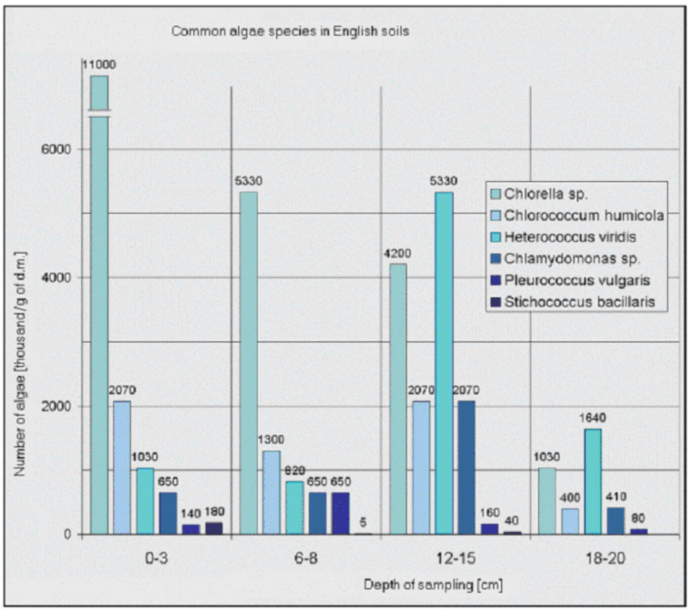

Fig. 1.6. Species of algae in English soils (in thousands/g of dry matter)

Algae
Algae live mainly in the upper layers of soil anywhere between 0-10 cm where the sunlight penetrates (rarely below 50 cm). Their number may vary between 100 thousand to 3 million per 1 g of soil (0.2 tons/ha). In favourable conditions, for instance in highly irrigated tropical soils, the numbers may be increased.

Soil fauna
Protozoa can widely develop in adequately humid soils. Their numbers reach anywhere from a few hundred to several million per 1 g of dry soil. The mass of protozoa in soil is between 0.1-0.5 tons/ha, whereas the mass of nematodes is between 0-0,2 tons/ha, earthworms 0-2.5 tons/ha, and other soil animals 0-0.5 tons/ha.

1.3. Edaphic factors

Soil is a natural living environment for various microorganisms as well as macroorganisms. Their development in soil depends on so called edaphic factors. It is the totality of factors that categorizes the soil such as: humidity, fertility (the accessibility of food elements in an available form), pH reaction and other physical factors that determine the development of living organisms in the ecosystems (Fig. 1.7).

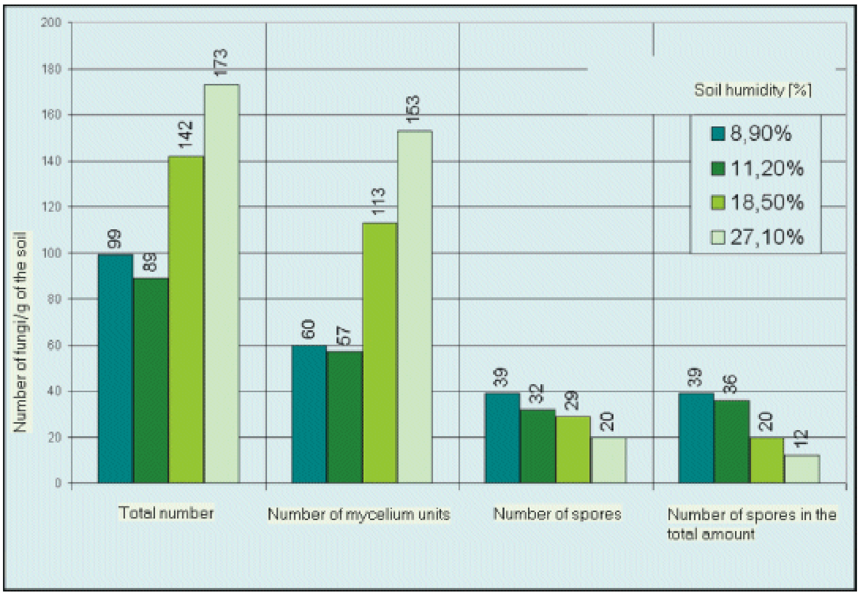

Fig. 1.7. Relation between humidity of the soil and number of fungi

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. At the same time it influences cell's maintenance of proper osmotic pressure and reaction.
- Also too high concentration of nutrients as well as excessive hydration slows down or stops all together the microorganisms' development.
- 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.
- Water strongly bonded with solid particles of soil may not be available for the microorganisms.
- 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 (WHC). Lower values cause decreased water activity, whereas the higher ones limit the degree of soil oxygenation.

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.

The Redox potential

- The Redox potential reflects the substances' tendencies to gain or lose electrons. It has a significant role in the course of physical and chemical reactions within the soil.
- 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 Fe^{3+}/Fe^{2+}, MnO_2/Mn^{2+}, So_4^{2-}/S^{2-} and on the level of oxygen within soil.

- The elements in the oxidized or reduced form create Redox patterns upon which the direction and character of metabolic transformation depends.
- Due to the dissociation processes, the soil's water has an influence on the value of the redox potential which in turn selectively affects the development and composition of soil microorganisms.
- 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.

Soil's 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 solution is characterized by buffer properties which give the ability to withstand reaction changes to a certain extent.
- 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 bacteria 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.

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 minus 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.

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' need for oxygen depends on the type of metabolism, the concentration and type of carbon and energy source, the phases of population growth and the physiological state of the cells. Only the bacteria can survive longer periods of time in anaerobic conditions.
- 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 O_2/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.

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.

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.

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.

1.4. Activity of microorganisms

Microorganisms reproduce and transform the organic matter creating biomass of their own cells and collect substrates essential for replenishing the supplies of humus. Additionally they decompose and mineralize the organic compounds, consequently recirculating the indispensable elements in plant production based on the assimilation of CO_2 from the atmosphere.

1.4.1. The role of microorganisms in organic metabolism – carbon cycle

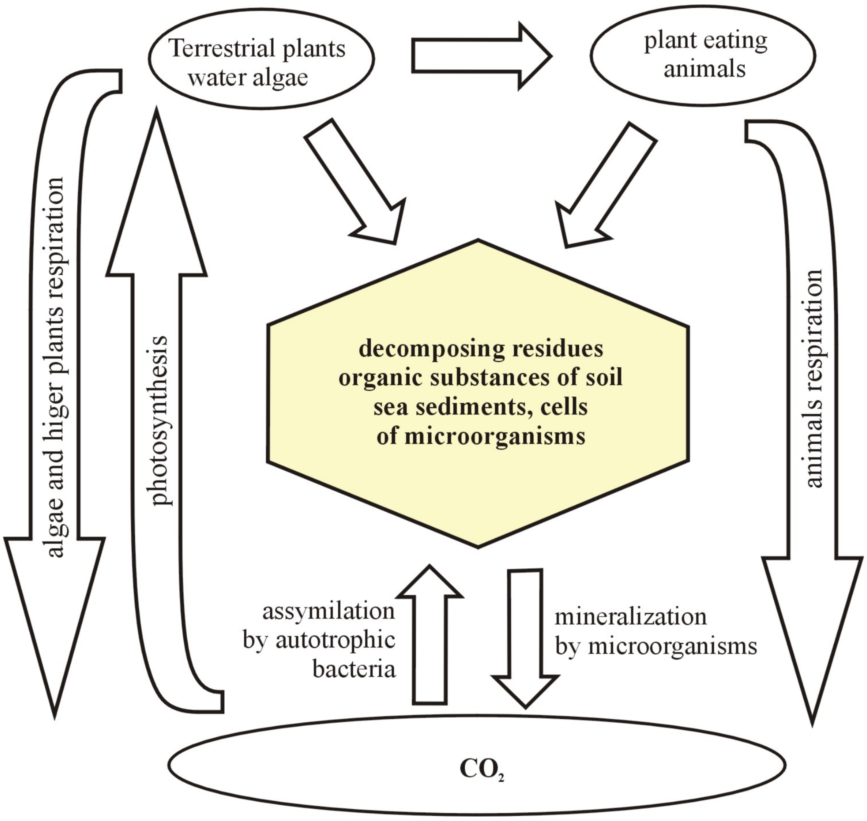

Fig. 1.8. The carbon cycle

- Carbon makes up 50% of mass of the organic matter that gets into soil in the form of plant and animal residues (falling leaves, various remains in meadows and forests, animal corpses, roots and shoots of dead plants).
- The fresh organic matter is composed of monosaccharides (hexose, pentose), polysaccharides (starch, cellulose, hemicellulose, chitin), organic acids, aromatic compounds (lignin, phenols, tannin), hydrophobic compounds (wax, cutin, fat and others).
- Carbon is recovered during the organic compounds decomposition and mineralization processes.
- Depending on its chemical nature, particular components of plants' mass are decomposed and mineralized at various speeds.
    a. Soluble substances such as sugars, amino acids, organic acids are easily washed away with water from plant and animal residues and then are quickly metabolized by soil microorganisms; they especially regulate the microbiological activities in the rhizosphere.
    b. Waxes, fats, rubbers and tannin are decomposed with great difficulty due to their high hydrophobic properties. Lignin is the most resistant substance to decompose among the plant materials.

Cellulose decomposition

- Cellulose occurs commonly in the walls of plant cells and is associated with hemicellulose and lignin. In the dry mass of green plants the content of cellulose is at 15-30% whereas in lignified parts and straw it can reach 50%.
- Cellulose is a polysaccharide that consists of a long unbranched chain of glucose units.
- Cellulolitic bacteria belong mainly to the genera: *Cytophaga*, *Cellfalcicula, Cellulomonas and Cellvibro.*
- The best known cellulitic system occurs in fungi. The *Trichoderma* genus releases the most active cellulase enzymes into the environment, then the enzymatic attack occurs away from the cells. Other fungi like *Chaetomium, Fusarium* and others also take active part in the above process.
- Decomposition may also occur in oxygen-free conditions-it's conducted by the genera: *Acetovibrio, Bacteroides, Clostridium, Ruminococcus.* Consequently a large number of gaseous substances such as CO_2, H_2, CH_4 are created.
- Decomposition of cellulose occurs faster in soils of neutral or slightly acidic pH and is slowed down in highly acidic soils.
- Microorganisms that decompose cellulose change it into simpler compounds and this way they create a nutrient base for all the soil heterotrophs

Lignin decomposition

Lignin belongs to a large group of aromatic compounds and, apart from cellulose, is a main component of wood tissues (up to 30% of plant biomass).

- Lignin is a polymer built of phenylopropane units which contain an aromatic ring and the methoxyl groups - OCH_3.
- The most active lignin degrading organisms are the fungi that cause so called white rot of wood. They decompose wood to CO_2 and H_2O. They belong to basidiomycete and ascomycyte groups and are represented by several hundred species. Among the basidiomycetes, the best known ones are: *Trametes versicolor, Phanerochaete chrysosporium*, oyster mushroom *Pleurotus ostreatus -* edible mushroom and *Lintunula edodes.* Among the ascomycetes the ones involved are: *Xylaria, Libertella and Hypoxylon.* Among the mould fungi *Trichoderma lignorum* demonstrates the ability to decompose lignin.
- The enzymatic complex composition that decomposes lignin is, among others, represented by oxidoreductases which require oxygen or hydrogen peroxide H_2O_2 for oxidative tearing up of bonds that connect phenylpropane subunits of lignin to each other. In direct decomposition of lignin the following takes part: laccase (may oxidize the monophenoles), lignin peroxidase and other enzymes that haven't been elucidated.
- The activity of microorganisms that decompose lignin in soil stimulates the production of humus.

Synthesis and humus decomposition

Humus is an amorphous organic substance, usually dark, that makes up the colloidal system of a large surface area capable of adsorbing ions of water and gases.

- It contains fractions of organic substances which have a low ratio of C:N (from 10 to 15), whereas the ratio of these elements in dead plants' residue is at about C:N=40:1.
- Fulvic, humic acids and humins make up the composition of humus. These are the conglomerates of more or less carbonized compounds, which are characterized by the presence of carboxyl, phenyl and methoxyl groups that contain C, O, N, P and S, as well as the aromatic skeleton with numerous side chains.
- The main humus forming system is the activity of soil microorganisms: bacteria (including actinomycetes) and fungi.

The synthesis of humus

- The process of humus formation is called humification.
- The main substrates from which humus compounds are formed are lignin, hydrocarbons and nitrogen compounds. However, the soil type and the climate conditions determine the kind of humus.

- Microorganisms conduct the following processes connected to the formation of humus:
  - they decompose fresh organic matter producing metabolites - the precursors of humus compounds.
  - they create biomass, which after atrophy and autolysis make up the additional initial substrates needed for the formation of humus.
  - they catalyze the processes of humus synthesis.

The basic way of humus compounds formation is through its synthesis from the fragments such as polyphenols with the participation of nitrogen components of protein origin.
The source of polyphenols may be the processes of lignin decomposition, hydrocarbon transformation and various microbiological synthesis processes. Many polyphenols form as the metabolites of different microorganisms.
The next stage of humification is the oxidation of polyphenols that leads to the formation of chinoid compounds. These transformations are catalyzed by the phenol oxidases produced by different microorganisms such as by the fungus *Serpula lacrymans*.
The final phase of the process is the polymerization of oxidized phenols.
Transformation of the organic matter in conditions when oxygen is available leads to the formation of humus and in oxygen-free conditions to peat deposit formation.

Decomposition of humus

- Degradation of humus occurs in conditions when there is a shortage of fresh organic matter and when there is not an adequate supply of nitrogen in soil.
- It is believed that the decomposition is caused by the autochthonous bacteria, which are adapted to the shortages of the available organic substances and they are consequently utilizing components contained in humus complexes. Particularly high degradation activity is demonstrated by the actinomycetes and some other bacteria such as *Micrococcus, Corynebacterium* and some white rot fungi such as *Polysticus*.
- It may be assumed, that both in humus formation and during its decomposition, the entire system of soil microflora and microfauna play a collective role.

1.4.2. The role of microorganisms in nitrogen processes in soil - the nitrogen cycle

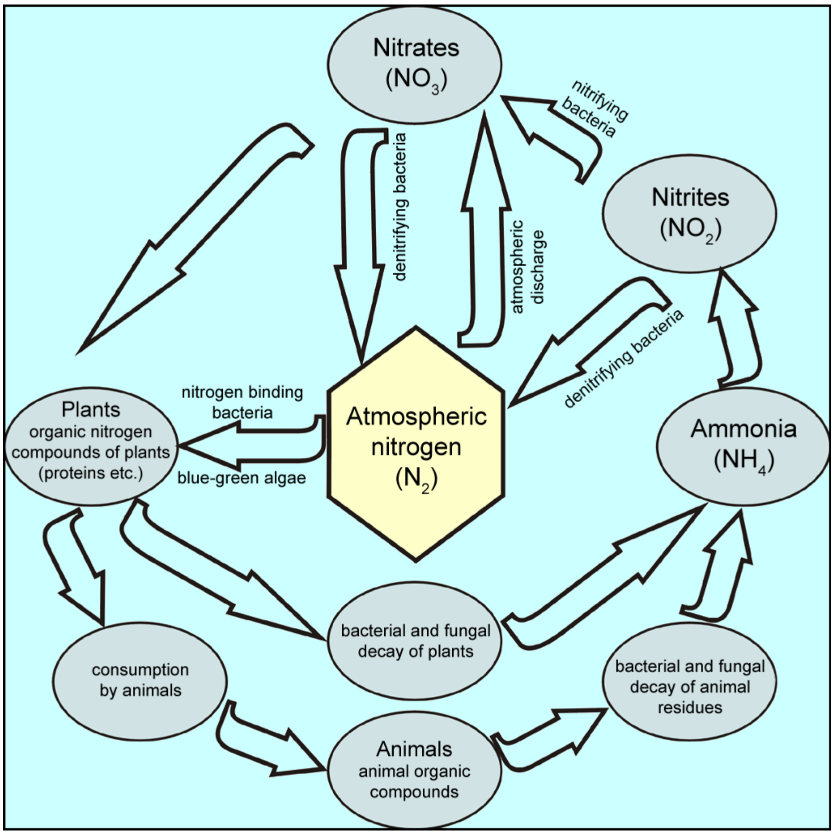

Fig. 1.9. Nitrogen cycle in soil

- Due to the microbiological processes nitrogen from the atmosphere is being incorporated into the compounds of the organic cells (so called nitrogen fixation)
- The organic compounds contained in animal and plant residues are mineralized by microorganisms and then are incorporated into the nitrogen cycle. In this way the free nitrogen level in the atmosphere is stable (78%).
- The Nitrogen cycle in the environment is composed of several links such as:
  - symbiotic and non-symbiotic fixation of atmospheric N_2 by microorganisms
  - microbial decomposition of the organic nitrogen compounds, ammonification - the release of NH_3 and of NH_4^+ions
  - the utilization of NH_4^+ ions for the re-synthesis of proteins by microorganisms. the utilization of NH_4^+ ions as ammonium salts by plants
  - the nitrification of NH_4^+ ions , nitrates are created through nitrites
  - the utilization of nitrates by higher plants as well as by some of the microorganisms (transformation of nitrogen into protein)
  - denitrification

Atmospheric nitrogen fixation

The assimilators capable of fixing of nitrogen only in symbiosis with plants

- *Rhizobium* bacteria living in symbiosis with papilionaceous plants supply soil with the most nitrogen (Fig. 1.10). They get into the root system of the plant where they multiply forming the long bacteria threads which penetrate plant tissue. The overgrowth of the plant tissue stimulated by bacteria causes the growth of nodules that form specific units upon the roots. Inside the nodules, a part of plant-infecting bacteria transform into bacteroids, which do not reproduce but are continuously active.
- It is believed, that the bacteroids take active part in the process of the atmospheric nitrogen fixation. The bacteroids contain a red dye called leghemoglobin. It is called that way because of the similarities to the hemoglobin. The iron Fe^{3+} contained in the dye is reduced to Fe^{2+}, thus it is believed, that the leghemoglobin may mediates the transfer of electrons to free nitrogen, hence causing its reduction.
- Inside the plant roots for instance of black alder root, nodules contain interacting actinomycetes (*Streptomyces alnii).*
- Nitrogen fixation by the free living bacteria is similar. The reduction of N_2 to NH_3 is performed by pyruvate dehydrogenase and nitrogenase enzymes.

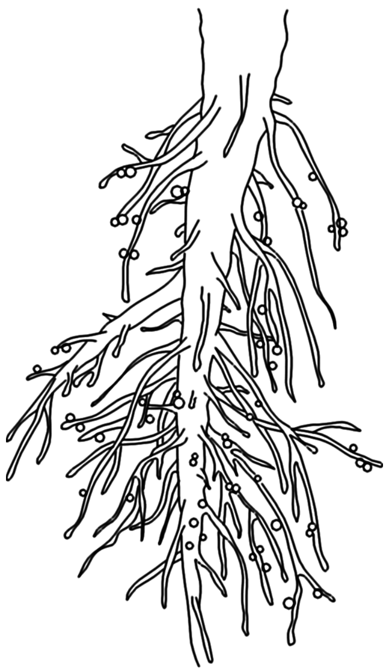

Fig. 1.10. A red clover root system with nitrogen-fixing bacteria *Rhizobium* sp.

Free living N_2 assimilators (non-symbiotic nitrogen fixation)
The following heterotrophic bacteria posses the ability to fix free nitrogen from the air and to enrich the soil with nitrogen:

- aerobes - *Azotobacter, Azotomonas, Derxia, Achromobacter, Beijerinckia*,
- microaerophiles – *Pseudomonas, Flavobacterium, Mycobacterium, Arthrobacter and Aerobacter*
- anaerobes - some species of *Clostridium* such as : *Cl. butyricum, Cl. pectinovorum*

Within the group of autotrophs the above capabilities are demonstrated by the photosynthesizing bacteria: *Chlorobium, Chromatium and* cyanobacteria e.g. *Anabaena, Nostoc.*

Ammonification
Ammonification is a process of the ammonium ion NH_4^+ or of the free ammonia formation. During the first stages of this process a break down of protein and a liberation of the amino acids occurs. Next, deamination of the amino acids takes place. The proteolytic break down of proteins occurs with a participation of the exocellular enzymes. Formed amino acids are transported to microorganisms' cells where the process of deamination takes place. Ammonia, being a gas, quickly spreads in dry soils whereas in humid ones it dissolves in water forming NH_4^+. Formed ammonium ions are utilized by the bacteria and plants for the synthesis of amino acids or undergoes the process of nitrification.

Nitrification
Nitrification is a biological process of oxidation of ammonia to nitrate accomplished by nitrificating bacteria (chemolithotrophs). The energy released during this process is utilized by bacteria in the synthesis of organic compounds.
The nitrification procedes in two stages:

- First, the ammonium is oxidized to nitrite, bacteria oxidizing NH_4 to NO_2 are described as the "nitroso": *Nitrosomonas, Nitrosospira, Nitrosocyjastis, Nitrosoglea*
- Second, the formed nitrite is oxidized to form nitrate. Nitrites are oxidized into nitrates by the group of bacteria called "nitro" such as genera *Nitrobacter, Nitrospira, Nitrococcus*
- The nitrifying bacteria are sensitive to the acidification of the environment; slowing down of their growth occurs at pH 5.0.

The nitrification process may be also conducted by the heterotrophic microorganisms. The biggest group that conductss the heterotrophic nitrification are fungi: *Aspergillus flavus, Penicillium, Cephalosporium.* The nitrification conducted by fungi is less sensitive to acidification and more resistant to drought. Formed nitrates in soil can be assimilated by the plants, flushed out by water or decomposed in the process of denitrification.

Denitrification
Denitrification is the process of nitrate reduction to form molecular nitrogen. The above process is conducted mainly in oxygen-free conditions and it is when the nitrates are utilized for respiration as the terminal electron acceptors. Several kinds of heterotrophic bacteria belonging to *Pseudomonas, Achromobacter, Bacillus, Micrococcus* genera are involved in the process of denitrification. The reduction of nitrates occurs in a few stages. During the first stage the nitrates are reduced to nitrites (NO_2^-), then the nitrites are reduced to nitric oxides (NO, N_2O) and down to molecular nitrogen. The process of denitrification is also conducted by some chemoautotrophic bacteria such as *Thiobacillus denitrificans.* The above bacteria obtain the energy from the oxidation of sulfur compounds to simultaneously reduce nitrates. Denitrification is believed to be a disadvantageous process since it leads to the deprivation of vital nitrogen compounds from plants. The loss of nitrogen from soil due to the denitrification increases with excess soil moistening, oxygen-free conditions, accumulation of nitrates and temperature increase.

1.5. Symbiosis forms

1.5.1. Mutual interaction between microorganisms

Symbiosis
Symbiosis *sensu stricto* is a constant or temporary interaction of two different species of organisms usually favorable and often essential for one or both of the partners.
The symbiosis in soil depends on a mutual utilization of the products of metabolism - one kind of microorganism may use the products made by other organisms.

- The example of the nitrification bacteria exemplifies the above. *Nitrosomonas* oxidize ammonium down to nitrites, however their high concentration in soil would be toxic for the above genus. Therefore, nitrites are being oxidized to non-toxic nitrates by the *Nitrobacter* genera. In the environment these bacteria always occur together.
- Another example of symbiosis are lichens. This is a simple form of interaction between algae (green algae or blue–green algae) and fungi (usually ascomycetes).

Parasitism
It is a peculiar type of microorganisms' interaction, in which representatives of one species use, for a longer period of time or permanently, other species as their living environment or (and)as a source of food.
Parasites may cause damage of whole populations of microorganisms. An attack of phages on nodule bacteria (*Rhizobium)* exemplifies the above.

Predation
It is a form of feeding when one species utilizes another living organism as a source of food. The best example of the above is the utilization of bacteria by protozoan organisms.

Antagonism
It is a mutual intolerance of different organisms in the same biotope.

- It may manifest itself in various ways, however most often it concerns competition for nutrient substances. Microorganisms that have a high requirement for food compete with microorganisms that have low need for nutrients. In such cases they deprive the "weaker" microorganisms of necessary nutrients consequently overrunning the habitat.
- The population of "less demanding" organisms triggers various defensive strategies. One way of eliminating the competition can be the accumulation of CO_2, acidification of the habitat or a production of growth-inhibiting toxins. In soil, a relatively high concentration of the following has a destructive impact upon all microorganisms: ammonium, nitrites and hydrogen sulfide.
- A peculiar variation of antagonism is the *antibiosis*. It is characterized by microorganisms releasing antibiotics as a by-product of dissimilation processes. The antibiotics are mainly produced by the actinomycets, rarely by other bacteria and fungi.
- Another variation of antagonism is the *mycolysis.* This variation is characterized by lytic influences of bacteria (including actinomycetes) on fungi with the help of enzymes such as cellulase and proteases (e.g. produced by *Pseudomonas and Bacillus).*

Commensalism
Commensalism is a kind of interaction between microorganisms, in which one organism benefits from another one without bringing any advantages or disadvantages to the other. For example, a process of stimulation of the other organism without the loss of its own growth. It can be manifested in various ways. Very often one group of bacteria modifies a substrate to form necessary products available by other groups.
For instance, a process of decomposition of cellulose by cellulose bacteria allows for the breakdown of this huge polymer into smaller particles that are utilized by other bacteria.

1.5.2. Mutual interaction of plants and microorganisms

The most typical example of direct interaction of bacteria with plants are the following symbiosis:

- bacterrhiza - the symbiosis of plants and bacteria
- mycorrhiza - the symbiosis of plants and fungi

The symbiosis of microbes and plants – bacterrhiza (Fig. 1.11)

- Symbiotic agreements are clearly exemplified in the rhizosphere, which is the area incorporating the outer surface of plants' roots and the adjacent soil.
- The symbiosis occurs when microorganisms settle in plants' roots systems. Both the plants and microorganisms may greatly benefit from such interaction.
- The best example of such interaction is the symbiosis of nodule bacteria *Rhizobium* with the papilionaceous plants.
- The symbiotic *Rhizobium* bacteria are among the best known nitrogen fixing organisms. *Rhizobium* belongs to the heterotrophic and aerobic bacteria. While developing inside the plant tissues they obtain the energy and carbon needed from its host. On the other hand the nitrogen assimilated from the air by the bacteria is utilized by the plant.
- The *Rhizobium* bacteria may live in soil for years without having any contact with a plant, by utilizing monosaccharides and mannitol as the source of C and energy. In such conditions they do not exhibit any abilities to reduce and fix nitrogen, but they obtain it from the substrate in the form of ammonium nitrogen. Nevertheless, once in the vicinity of a papilionaceous plant, with which they can interact, they penetrate its root system and form nodules that participate in atmospheric nitrogen fixation.
- The symbiotic system is formed only with particular types of papilionaceous plants and the suitable species of *Rhizobium* genus.

Figure 1.11. Examples of the symbiosis of a plant (vetch *Vicia L.*) and bacteria

Rhizosphere

- The rhizosphere is the layer of soil around the roots where among others, in great concentrations, live bacteria, fungi, protozoa, nematodes, mites, springtails, which usually form groups of species characteristic to a given plant.
- The rhizosphere is occupied by a large variety of forms, however the *Pseudomonas* and *Achromobacter* as well as the denitrifiers are the most numerous, and less numerous are the *Arthrobacter and Bacillus* forms. The above organisms utilize nutrients released by the roots. The increased number of microorganisms is

accompanied by higher activity of soil's fauna, especially of those organisms which feed upon roots and microorganisms.

- The number of bacteria in the rhizosphere may even be 1000 times higher than outside the rhizosphere. The ratio of bacteria from within the rhizosphere to the number of bacteria from outside is called the rhizosphere effect and it is marked with the R/S symbol (R – rhizosphere, S – soil).
- Microorganisms of the rhizosphere also have a big affect on plants. They lead to a continuous breakdown of organic and mineral compounds, which become available to plants. Moreover, they produce organic and non-organic acids, influence the dissolution of mineral salts and protect the plants against the phytopathogens.
- An unfavorable effect is due to the metabolic activity of microflora that cause the depletion of the oxygen required for development of denitrifiers. As a consequence, some phytotoxic substances may be produced, like alcohols, antibiotics or phenol compounds.

The symbiosis of fungi and plants

Mycorrhiza is the interaction of fungi with vascular plants. In this type of interaction both organisms benefit. Fungi grow into plant roots. They penetrate its cells and stimulate their growth by producing auxin hormone. Due to mycorrhiza, plants obtain larger absorbing surface and better access to nutrients being broken down and absorbed by fungi. Plants supply fungi with organic substances in the form of assimilation products transported from leaves to roots. Mycorrhiza is a wide-spread phenomenon; it concerns not only roots of trees, bushes, species of flowers but also cultivated plants such as grains and potatoes. There are two types of the mycorrhiza:

- ectotrophic,
- endotrophic.

Ectotrophic mycorrhiza

- Fungus develops upon the surface of plant roots, creating a kind of muff composed of intertwined threads of mycelium. The outer hyphae of this mycelium penetrate the soil, while the inner ones penetrate the surface layers of root tissues.
- As the result of the mycorrhiza, roots lose their root hairs and become shorter since the functions of roots are taken over by the fungi. Because mycelium's suction force is much stronger than that of the roots the plant is better supplied with water and mineral salts than in the cases when there is no mycorrhiza.
- For plants interacting with mycorrhizal fungi, the absorption of nitrogen may increase by 90%, phosphorus by 20% and potassium by 75%.
- Fungi also produce substances that stimulate roots' growth, and some are able to fix nitrogen.
- That kind of symbiotic fungi mainly belongs to the *Basidiomycetes.*

Endotrophic mycorrhiza

- Endotrophic mycorrhiza occurs usually among green plants and some deciduous trees.
- In this type of interaction the plant roots do not differ externally from those without mycorrhiza.
- The inside of the root cells is filled with a thick network of intertwined hyphae which are partially digested by the plant.
- Endotrophic mycorrhiza is formed by the *Fungi imperfecti.*

1.6. Soil bioremediation

How we can define bioremediation?

Bioremediation is a group of treatment methods or processes designed to enhance the natural microbial degradation of organic contaminants. The microorganisms carry out the degradation of harmful substances to a less toxic or non-toxic state. Microorganisms utilize the environment-polluting organic compounds as food substrates. After the degradation of polluting compounds the population of microorganisms reduces. Dead microorganisms or low numbers of microorganisms once lacking food substrates no longer pose any danger to the environment.

Bioremediation criteria

The goal of bioremediation is the neutralization of the organic pollution to achieve undetectable concentrations or concentrations permissible by the national regulations of particular countries. Bioremediation is utilized for cleanup of grounds and ground waters as well as sewage and sludge.
During the utilization of bioremediation for the purpose of pollution neutralization the following conditions must be met:
a) the environment undergoing bioremediation should contain microorganisms characterized by the specific catabolic processes,
b) microorganisms utilized within the bioremediation process should be capable of efficiently converting chemical compounds and reducing their concentration down to the level allowed by the regulations,
c) metabolites produced during the biodegradation can not have toxic, mutagenic or carcinogenic properties,
d) the conditions in the immediate area where the process is being conducted should be favourable to the growth and activity of the microorganisms (adequate nutrients, acceptable pH, oxygen or other electron acceptor, acceptable redox level, favourable moisture)

The rate of biodegradation may be limited by: temperature, the toxicity of concentrated contaminants; or mass transfer limitations.

Fig. 1.12. Soil contaminated with petroleum products

1.6.1. Microorganisms used in remediation technologies

The bioremediation processes may be conducted by the autochthonous microorganisms, which naturally inhabit the soil/water environment undergoing purification, or by other microorganisms, that derive from different environments. However in both cases they are characterized by a high ‘xenobiotics' degradation activity (Fig. 1.13).

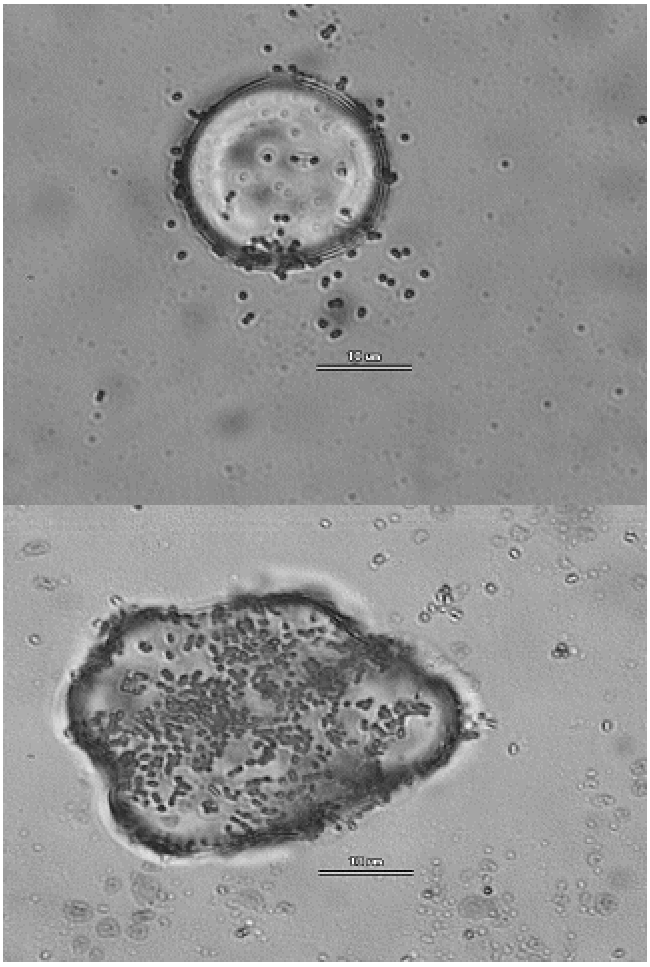

Fig. 1.13. Diesel oil droplets colonized by bacteria

The choice of strains capable of being used for the inoculation of the contaminated grounds creates many problems.

- Apart from the high efficiency in ‘xenobiotics’ decomposition, the chosen strains should also possess many additional features, that enable their adaptation and development in a new environment.
- One of the conditions for the adaptation of the inoculants in soil is a lack of antagonistic interaction with the natural water and soil microflora.
- Moreover, they can not be pathogenic microorganisms nor ones, which, during growth on hydrocarbons, produce substances with cytotoxic, mutagenic or carcinogenic properties.

The selection of microorganisms specialized in degradation of particular compounds is based on the processes of their adaptation or genetic operation. Substances making up the environment's contamination, as a rule, are composed of many compounds. Therefore, a complete soil cleanup requires a special, carefully selected, mixture of microorganisms. Utilization of mutated microorganisms is useful only in *ex situ* methods where precise conditions of the process control and the prevention of contamination by dangerous mutants exists. In order to avoid problems related to the introduction of foreign organisms into the environment, mixtures of microorganisms should be created to degrade the contamination. Moreover, the techniques used to allow microorganisms adapt to new conditions should constantly be improved.

The initial high number of microorganisms in soil may be obtained at the beginning of the purification process by the inoculation of the ground with microorganisms. This allows a faster and more effective bioremediation process. Inoculation of the soil is carried out after growing the microorganisms in a bioreactor (Fig. 1.14). The cultured natural autochthonous microorganisms or specially prepared strains derived from a culture collection and/or the commercial preparations are utilized in the process of inoculation. The strains are stored in the lyophilised form, frozen or placed in a special suspension. The microorganisms may be incorporated into both the soil and the water environment in the form of a suspension or placed on a solid support (immobilized).

Fig. 1.14. Bioreactors with aeration and temperature control systems

The microbial cultures usually contain a mixture of bacteria, nutrients, a solid support and possibly enzymes. Depending on the composition of these bio-preparations used for bioremediation the above can be subdivided into microbiological (bacterial), enzymatic and bacterial–enzymatic. The advantage of microbiological preparations in relation to enzymatic preparations is the fact that the microorganisms multiply in a previously clean environment whereas the enzymatic preparations are added, in specific dosages, without the possibility of multiplication.
One of the conditions which have to be met in order to obtain effective biodegradation is the bacteria's accessibility to the contaminated layers of soil. Migration within the soil depends on the number of microorganisms as well as on the type of soil.
The effects of the biodegradation process of xenobiotics in soil are dependent on the method used to inoculate the soil with microorganisms. It has to provide an even distribution of microorganisms within the soil and contact with nutrients contained in the soil.
Surface introduction of the inoculants is not highly effective due to slow migration of microorganisms, especially in soils, which contain clay and silt. In order to speed up the process of migration the following are applied:

- injections with the use of high-pressure equipment,
- immobilization of microorganisms on solid supports,
- electromagnetic field to speed up bacterial migration in the soil.

Due to the fact that bio-preparations are composed of living organisms, their application requires thorough knowledge of the subject as well as a careful supervision of the cleaning process.

1.6.2. Stimulation of bioremediation

Bioremediation processes are designed to optimise the conditions for microbial growth and degradation of contaminants. Microbial growth and metabolism of contaminants require macronutrients (phosphorus and nitrogen) and micronutrients.

The demand for nutrients is specific for each case. It has been determined that the ratio of carbon to nitrogen and phosphorus within the soil should range from 100:10:1(by weight). Therefore, the basic condition for a proper bio-stimulation is the control of nitrogen and phosphorus concentrations in the soil by application of mineral fertilizers. The most suitable ones, for the above purpose, are ammonium sulphate and sodium phosphate (sources of nitrogen and phosphorus). Moreover, magnesium sulphate, sodium carbonate, calcium chloride, iron sulphate are also used for the purpose.

The choice of appropriate dosages of the biogenic substances ought to be very precise and readjusted to the soil conditions, since it has been shown that, for example, nitrogen compounds in excessive numbers may slow down the biodegradation process.

The selection of nutrients with an optimum composition is conducted in laboratories. The type of missing nutrients is established by growing bacteria in the presence of various sources of biogenic elements and by careful supervision of their growth. In addition, pollution reduction, oxygen consumption and dioxide release are measured.

1.6.3. Classification of bioremediation methods

The division of bioremediation methods may be done in accordance with the level of environment oxygenation as well as the location of the cleaning process.

Depending on the level of environmental oxygenation the types of biological purification can be divided into aerobic, anaerobic/aerobic, and anaerobic. Aerobic microorganisms use oxygen as an electron acceptor. Anaerobic microorganisms use other electron acceptors such as nitrate, sulphate, iron, manganese, or certain organic compounds.

Facultative organisms can use oxygen, if it is present or other electron acceptors if it is not. Most soil bioremediation processes are aerobic. In anaerobic conditions the microbiological decomposition of pollutants is slower and the end products may have a toxic character.

The source of oxygen utilized by microorganisms in the respiration processes may be the atmospheric air that gets into the soil passively or by force. The passive route depends on natural ground penetration by the atmospheric air. Active increase of the delivered oxygen can be done by mechanical mixing of the surface layers of soil (harrowing, ploughing etc.), the introduction of special perforated spray lances driven directly into the soil or aeration with compressors and fans (Fig. 1.15-16). In addition, the environmental enrichment in oxygen may take place because of the utilization of hydrogen peroxide, which breaks down in soil to water and oxygen.

Depending on the level of contamination as well as the character of the re-cultivated environment, bioremediation may take place through both, *in situ* or *ex situ* methods. In the first one, *in situ*, the point is that the pollution is eliminated directly at the place where it occurs. However, in the second method the contaminated ground or waters are excavated before the actual regeneration procedures. *In situ* techniques do not require excavation of the contaminated soils so may be less expensive, create less dust, and it is possible to treat a large volume of soil and cause less release of contaminants than by *ex situ* methods. But *in situ* techniques are slower than *ex situ*, may be difficult to manage, and are most effective at sites with permeable soil. *Ex situ* methods are utilized where there is danger of toxic pollutant migration into the ground waters and when the process of detoxification must be conducted in a short period of time.

Fig. 1.15. Passive ventilation of the excavated ground using air lances

Fig. 1.16. Active aeration system

1.6.4. In situ methods

In situ methods are based mainly on bio-stimulation of the organic pollutant degradation processes by enriching waters or soils in biogenic elements, by acidity correction as well as by their aeration. The following *in situ* methods can be used.

Agricultural methods

Those are the most commonly used methods of soil decontamination. They are effective with almost all components of fuels. Lighter products, such as gasoline, are eliminated by vaporization or, to lesser extent, by biodegradation. When it comes to heavy products, like diesel fuels and kerosene, contamination is eliminated mainly by biodegradation. There are many variations of agricultural methods depending on the technical solutions. The simplest variation depends on spreading the contaminated soil in a thin layer of no more than 0.5m thick followed by a period ploughing or deep harrowing in order to

aerate the soil. This activates the micro-flora by delivering essential nutrients, oxygenating the soil and by de-acidification when needed.
Nutrients are replenished by application of nitrogenous, phosphate or potassium fertilizers when necessary. Soil de-acidification can be accomplished by liming. Planting grasses or papilionaceous plants on the contaminated grounds may also assist the cleaning processes. Detailed programs for fertilization, mechanical and other treatments are designed for each individual case.

Bioextraction

The situation is significantly more complicated when the need for removal of contaminants from within deeper layers of soils arises. The growth stimulation of microorganisms decomposing for example, petroleum products is more difficult; nevertheless the methods are similar. It is also essential in this case to deliver missing nutrients, supply oxygen and, if necessary, inoculate the soil with microorganisms able to actively decompose the contaminants. The processes of soil venting and rinsing with solution containing nutrients or active bacterial cultures are utilized as well. For the above, the following technical equipment is necessary: suction and positive displacement pumps, sumps, and screens preventing the spreading of contaminant within soils. The condition of an effective cleaning process is also a proper geological configuration of the terrain, which allows a controlled flow of the medium (air, water vapour, solutions).

The natural decomposition can be accelerated by utilizing the so-called bio-extraction. Optimization of the process can be accomplished by rinsing the soil and water environments through forced infiltration of ground water (water stimulated bioremediation *in-situ*) and aeration (soil bio-ventilation). Microorganisms and nutrients delivered with water stimulate biodegradation of petroleum products while the air enriches soil in oxygen thus assisting in the biodegradation.

Water stimulated bioremediation

The goal of the above process is to force a vertical and then horizontal flow of water along with the organic contaminants in the water and soil environment (Fig. 1.17).

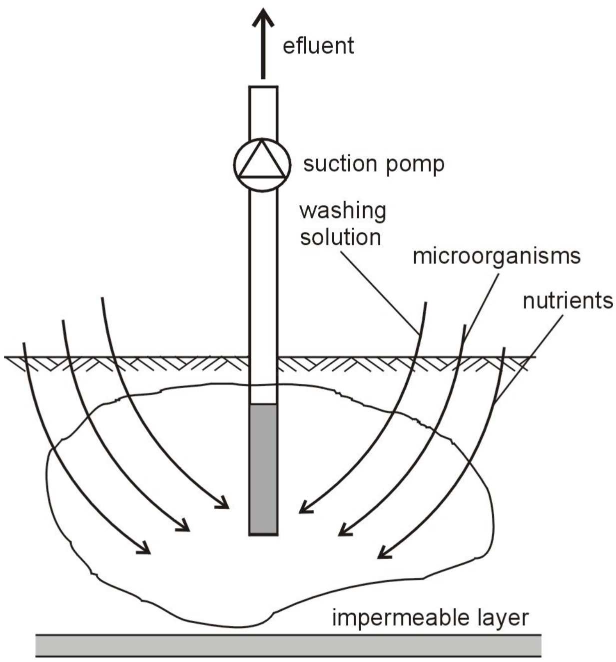

Fig. 1.17. Water stimulated soil bioremediation

This method is utilized for the cleaning of the environment from petroleum related non-soluble substances gathered at the surface of ground waters. During the process the ground water is pumped out up to the surface, purified, oxygenated, and returned back to the source after nutrient enrichment. In other cases, the flow of the underground waters may be utilized. The waters are obtained from wells, which are situated in the lowest points.

These waters are then sent to bio-reactors, in which the process of biodegradation of petroleum-related products occurs. The same water is then returned back to its source. The above method is a combination of *in situ* and *ex situ,* methods which are complementary to each other, which in turn, allows for optimisation of the process. Water stimulated bioremediation can be assisted by surfactants. It has been shown that synthetic surfactants and biosurfactants accelerate the processes of hydrophobic contaminants biodegradation, particularly of heavy fractions of petroleum products. Increased solubility and emulsion formation result in better mobility of petroleum products in soil and in larger specific surfaces accessible to microorganisms. Surfactants can also increase the permeability of soils

Bioventilation

The acceleration of the natural processes of biodegradation may be assisted by soil ventilation. Ventilation is a physical method, which may be utilized as an independent decontamination technique used for the maximization of volatilization of low molecular mass hydrocarbons (for example products based on gasoline or solvents). However, during this process a very little biodegradation occurs.

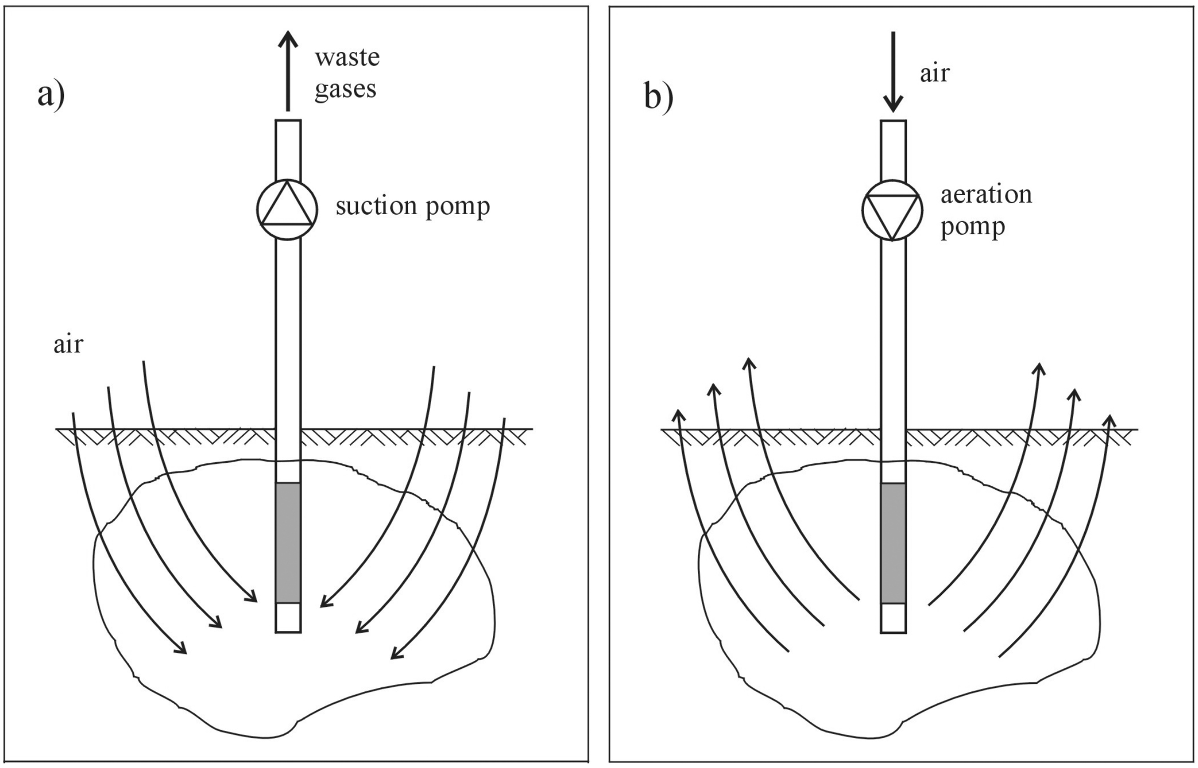

Fig. 1.18. Bioventilation
a) extraction of the ground air b) air injection

The ventilation process permits the removal of volatile petroleum products from the aeration and saturation zones, at the same time enriching the ground with air and increasing the level of oxygenation.

The aeration process can be both, active or passive. In the case of passive ventilation the aeration is done by the utilization of perforated pipe networks. Active ventilation however, depends on the creation of negative pressure (extraction of the ground air) or positive pressure (air injection) (Fig. 1.18).

The effectiveness of the soil and water environment bio-ventilation depends on the level of oxygen in the air contained in the soil, level of bio-genes, reduction-oxidation conditions, presence of surface-active agents, level of saturation (moisture), pH and temperature.

The most effective way of supplying oxygen to the contaminated ground is by introducing it with compressed air. Ozone (O_3) is also utilized as an alternative source of oxygen. However, there is some danger in using ozone as the source of oxygen since it possesses some toxic properties.

1.6.5. Ex situ methods

Ex situ methods can be implemented when the danger of toxic pollutant migration into the ground waters occurs and when the process of detoxification has to be completed in a short period of time.

Bioreactor method

The decontamination process is most effective when run in specially designed bio-reactors (Fig. 1.19). They provide effective control of all parameters, delivery of required ingredients, oxygenation and inoculation of the soil. The cost of this method is however rather high due to the technological requirements as well as the need for transportation of heavy masses. Therefore, the above method is not often used and is usually for smaller amounts of soil.

Among the technologies based on *ex situ* methods there are bio-reactors and lagoons used for decontamination of oily sewage waters and reactors for treatment of semi-liquid masses of contaminated soil, sludge and deposits. Solid materials undergo mixing with liquids followed by aeration.

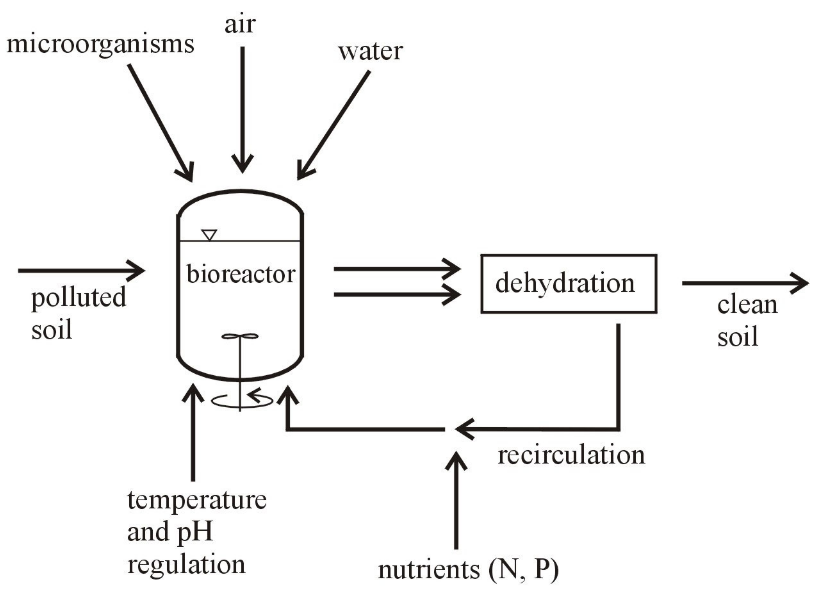

Fig. 1.19. Schematic of the bioreactor method

In both types of bioreactors the level of dissolved oxygen may be controlled just like the pH and the concentration of inorganic nutrients. This technique is similar to the one that treats municipal wastes by utilizing the activated sludge method.

Moreover, the technique may be aided by surfactants or dispersants in order to desorb the hydrocarbons from the solid particles, and to increase the degree of dispersion of water insoluble oil contaminants. Surface-active agents used in these methods may be synthetic or natural. Bio-surfactants* have found ever-increasing use due to the fact that they are more environmentally friendly and are biodegradable.

Bioreactors may also be utilized for decontamination of ground waters. They are either stationary or portable, which makes the process easier and less expensive. Portable bio-reactors are used for decontamination of ground waters after pumping out the material from the water-bearing bed or for decontamination of waters derived from the process of contaminated ground rinsing. There are two different types of bioreactors, fluidized and immobilized beds. The choice of bed depends mainly on the type of contaminants and their concentration in water that is going to be processed. The immobilized biomass is a mixture of selected microorganisms capable of biodegradation of particular pollutants. The microorganisms may be trapped inside the carrier's structure (natural polymers such as agar, alginate, collagen, or synthetic polymers such as polyacrylamide gels, polyurethanes and others) which is called active immobilization.

In the passive immobilization microorganisms are bound to the surface of a porous material (activated carbon for instance) or may create a gelatinous film upon the surface of stationary elements (ceramic rings, plastic tiles, polyurethane foam). In such bio-reactors the contaminated water flows countercurrent to the air through the solid bed containing the immobilized microorganisms.

Biopile method

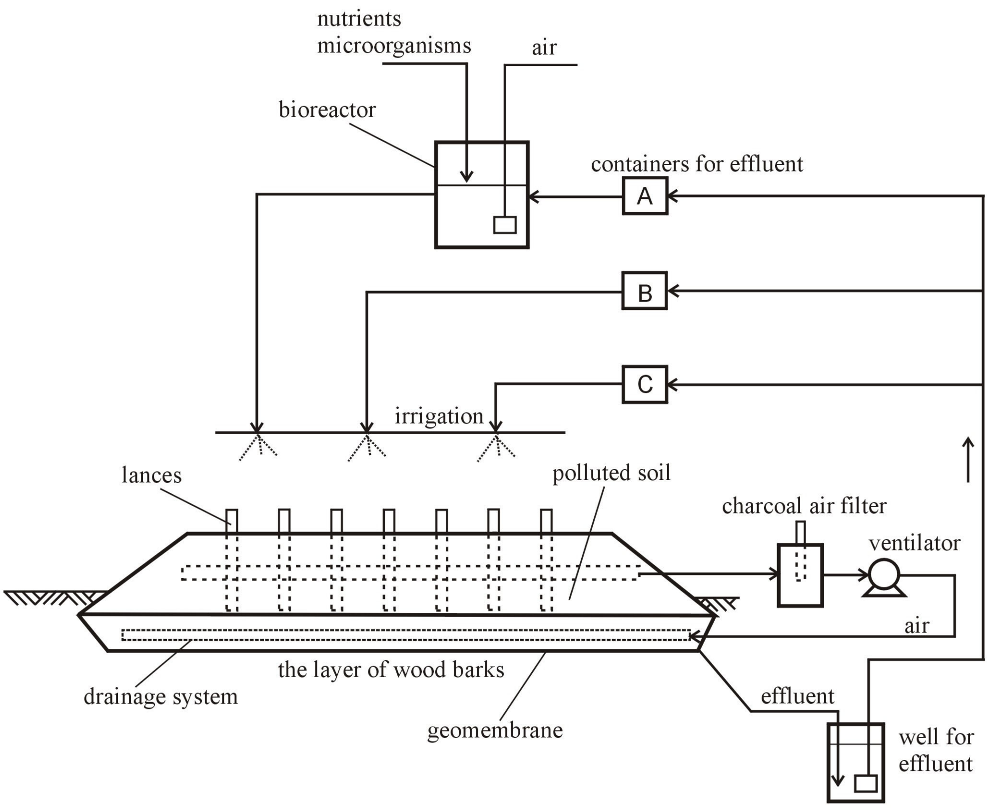

Fig. 1.20. Scheme of biopile method

Fig. 1.21. Forming the pile with the drainage system

The method consists in transferring the contaminated soil or ground into a specially prepared place (Fig. 1.20-21). The excavated soil is arranged in the form of an elongated heap inside a foil-lined ditch that is equipped with drainage and ventilation systems.

The process is also aided by aeration as well as water and nutrients addition. The air is pumped in through the system of perforated pipes equipped with a blow-fan that creates negative pressure at the bottom of the heap. In simpler technologies the actual aeration is ensured by mechanical shifting of the soil. The heap is usually covered with a foil tunnel equipped with a system of sprinklers through which water and nutrients are delivered. In many recently used technologies water circulates in a closed system (Fig. 1.20). Reflux from within the ground is filtered into bioreactors and then pumped into the reservoir from which, after enrichment in nutrients and growth of the microorganisms, it is returned by the sprinkler system onto the heap.

Evaluation

Can you specify what kind of microorganisms live in soil?
What is the role of bacteria in the soil environment?
Is lignin decomposed in the soil?
Can you trace the sequence of events in the nitrogen cycle and list the names of microbes that play a key role in this cycle?
Explain the significance of mycorrhizal associations
How do microbes influence soil fertility?
What type of microbes play an ecological role as decomposers?
What is the rhizosphere?
What is the difference between mycorrhiza and rhizosphere?
What limits the bioremediation?
What types of microorganisms are used in bioremediation process?
Can you explain the difference between *in situ* and *ex situ* methods of bioremediation?

Summary

The soil is composed of mineral and organic particulate solids, air, soluble components, and living organisms - edaphon. Microorganisms such as viruses, bacteria, fungi and protozoa are the most important components of soil edaphon.
Microorganisms process the organic matter creating biomass of their own cells and collect substrates essential for replenishing the supplies of humus. Additionally, they decompose and mineralize the organic compounds, consequently recirculating the indispensable elements in plant production that are based on the assimilation of CO_2 from the atmosphere.
It is possible to remove pollutants from soil by using biological methods. They are economic and environmentally friendly.

Glossary

Edaphic: (i) Of or pertaining to the soil. (ii) Resulting from or influenced by factors inherent in the soil or other substrate, rather than by climatic factors.
Fulvic acids: Yellow organic material that remains in solution after removal of humic acid by acidification.
Humus: Total organic compounds in soil exclusive of undecayed plant and animal tissues, their "partial decomposition" products, and the soil biomass. The term is often used synonymously with soil organic matter.
Humic substances: Series of relatively high-molecular-weight, brown-to-black substances formed by secondary synthetic reactions. The term is generic in a sense that it describes the coloured material or its fractions obtained on the basis of solubility characteristics, such as humic acid or fulvic acid.
Humification: Process whereby the carbon of organic residues is transformed and converted to humic substances by biochemical and chemical processes.
Mycorrhiza: The symbiotic association between specific fungi with the fine roots of higher plants.
Rhizosphere: Zone of soil immediately adjacent to plant roots in which the kinds, numbers, or activities of microorganisms differ from that of the bulk soil.
Root nodule: Specialized structure occurring on roots, especially of leguminous plants, in which bacteria fix nitrogen and make it available for the plant.
Symbiosis: The living together and intimate association of two dissimilar organisms.
Xenobiotic: Compound foreign to biological systems. Often refers to human-made compounds that are resistant or recalcitrant to biodegradation and decomposition

2. Microbiology of water

Contents

Aims

During the study of this chapter you should gain knowledge about water biocenoses and factors limiting their development. In particular, you should be familiar with microorganisms which have a significant influence on the energy and matter recycling process in that ecosystem. This chapter will give you key information about microorganisms living in polluted water and processes which are carried out by them. The dangers associated with pathogenic organisms transmitted by water and methods of controlling the water sanitary quality are discussed. Finally, biological methods of wastewater purification are reviewed.

Orientation

In this chapter we first characterise the water environment and factors which influence the activity and development of water organisms. After that, the water microorganisms are discussed. The second part of this chapter deals with polluted waters, water borne pathogenic organisms and water health standards. Finally, the possibilities to remove pollution by biological methods are discussed.

Prior knowledge

What can you say about water chemistry? Do you know something about the cell structure of prokaryotic and eukaryotic organisms? What is their metabolism like?
If unsure, refer to Chapter 1.

Study advice

First you have to read some short information about water and groups of organisms'that inhabit that ecosystem. If you have any problem with the remaining water chemistry you have to return to textbooks. Following this you can read about the characteristics of water microorganisms. For a good understanding of the microbiology of clean and polluted waters, a basic knowledge about cell structure and metabolism is necessary.

2.1. Water

What is water?

Clean water is a colourless, tasteless and odourless liquid that has a boiling point at 100°C and freezes at 0°C (under the pressure of 760 mm Hg). Water occurring in nature contains dissolved salts and gases, especially sea and mineral waters. Water covers 70% of the earth's surface, and thus, it is the most essential habitat of life. The overall volume of inland waters is estimated at 7.5×10^5 km ^{3}, of seas and oceans at 1.4×10^9 km^3, and of glaciers and continental glaciers at 1.8×10^7km.
Water makes up the most crucial component of living organisms (70-90% of cell mass) and fulfils a purpose in taking part in various biological reactions and processes.

What types of waters are inhabited by microorganisms?

The biotopes of water microorganisms may be underground and/or surface waters as well as bottom sediments.

- The underground waters (mineral and thermal springs, ground waters) - due to their oligotrophic character (nutrient - deficient) are usually inhabited by a sparse microflora that is represented by a low number of species with almost a complete lack of higher plants or animals.
- The surface waters such as streams, rivers, lakes and sea waters are inhabited by a diverse flora and fauna. Microorganisms in those waters are a largely varied group. Next to the typical water species, other microorganisms from soil habitats and sewage derived from living and industrial pollution occur.
- Bottom sediments are a transient type of habitat i.e. the soil-water habitat that is almost always typically oxygen-free in which the processes of anaerobic decomposition by microorganisms cause the release of hydrogen sulphide and methane into water. In the bottom sediment, anaerobic putrefying microflora, cellulolytic bacteria and the anaerobic chemoautotrophs develop.

2.2. Groups of water organisms

Microorganisms occupy surface waters in all of the zones; they may be suspended in water (plankton), cover stationary underwater objects, plants etc (periphyton), or live in bottom sediments (benthos).

2.2.1. Plankton

The group of organisms that passively float in water not being able to resist the movement and the flow of water mass is called plankton or bioseston.
We differentiate:

- phytoplankton (plant plankton)
- zooplankton (animal plankton)
- protozoa plankton
- bacterioplankton (bacteria plankton)
- virus plankton

Phytoplankton - are mainly microscopic algae and blue-green algae. It is a varied community in terms of the systematics and mainly composed of forms smaller than 50μ m. Sea phytoplankton are dominated by diatoms and dinophyta, whereas fresh water phytoplankton are dominated one by cryptophytes, diatoms, green algae, and blue-green algae.

Zooplankton - are small water animals that occur in plankton. There are three systematic groups that occur in fresh waters: rotifers, branchiopods and copepods. The sea water plankton is composed of copepods, ctenophores, urochordata, arrow-worms as well as some species of snails. Most of them are filtrators (condense suspended particles) or predators.

Protozoa plankton consist of protozoa which occupy the open water zones like flagellates and ciliates. They are the main consumers of bacteria. Moreover, most ciliates feed upon flagellates, algae and smaller ciliates. The protozoa itself feeds the zooplankton.

The heterotrophic **bacteria plankton** occupy waters which are abundant in organic compounds. The amount of bacteria in open waters varies between 10^5-10^7 cells in 1ml.

Virus plankton is composed of viruses which are the smallest element of plankton. Their numbers may be very high (from 10^8 in 1ml) in various fresh and sea water habitats. Viruses are, next to the protozoa, a crucial factor in bacteria mortality.

Distribution of plankton

The ability to hover in mid water is possible due to the presence of mucous membranes around the cell, gas vacuoles or lipids contained inside the cells.
The distribution of species and numbers of water organisms differs greatly since the biotic and abiotic factors vary in particular water basins.
The distribution in lotic waters such as rivers, springs and streams is more or less the same. Especially high numbers are found in the mid course of the river where the bottom and the main-stream speed are favourable for development of lakes. Where the flow of water is limited, a closely related vertical distribution of mainly phytoplankton to stratification has been observed.
During calm, quiet weather where the air meets the water, neuston appears upon the surface of the water. This is composed of bacteria, algae and pleuston which is composed of larger organisms (Fig. 2.1).

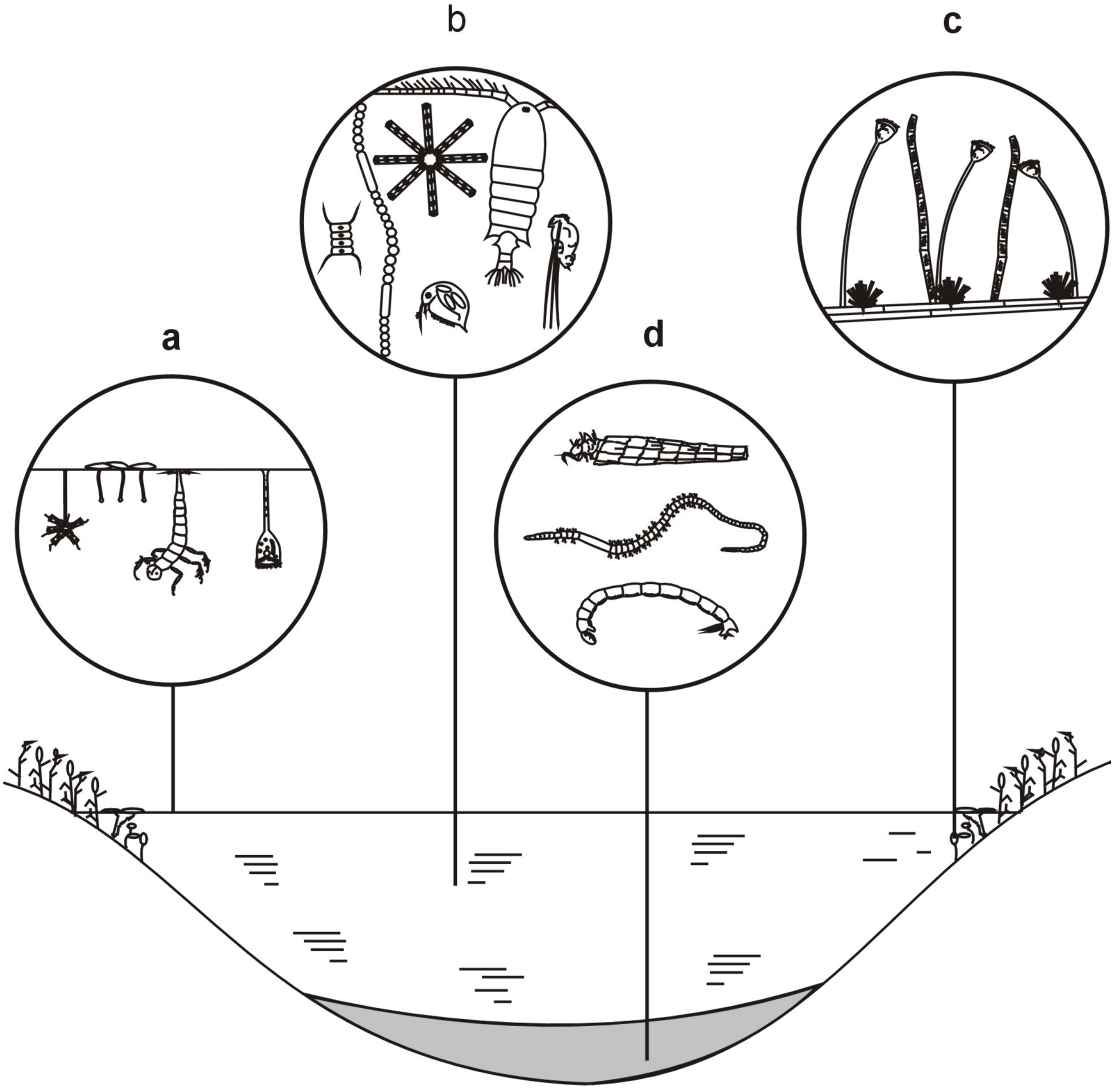

Fig. 2.1. Groups of organisms living in lakes:
a) pleuston and neuston, b) plankton, c) periphyton, d) benthos

2.2.2. Periphyton

Periphyton occupy the shore line zones. They are a group of organisms that create outgrowths upon various objects and underwater plants. Most of the time they usually consist of small algae - diatoms, green algae and bacteria. Moreover, various settled or semi-settled protozoa, eelwarms, oligochaetes, insect larva, and even crustaceans make up the periphyton biocenosis. Periphyton has a characteristic complex biocenosis and many ecological relationships can be observed between its components.

2.2.3. Benthos

The bottom habitat is occupied by a group of organisms called the benthos (Fig. 2.2). The muddy bottom contains an abundance of organic compounds that are created as a result of dead matter decomposition (fallen parts of plants and animals). At great depths the bottom is free from any plants which, due to a lack of light can not grow. However, the absence of oxygen supports the development of, among others, an oxygen-free putrid microflora. Among the benthos microflora the most numerous are bacteria and fungi (decomposers) as well as some animals (detritophages). Both of the above groups are responsible for decomposition of the organic matter. Benthos of shallow reservoirs may also contain some algae.

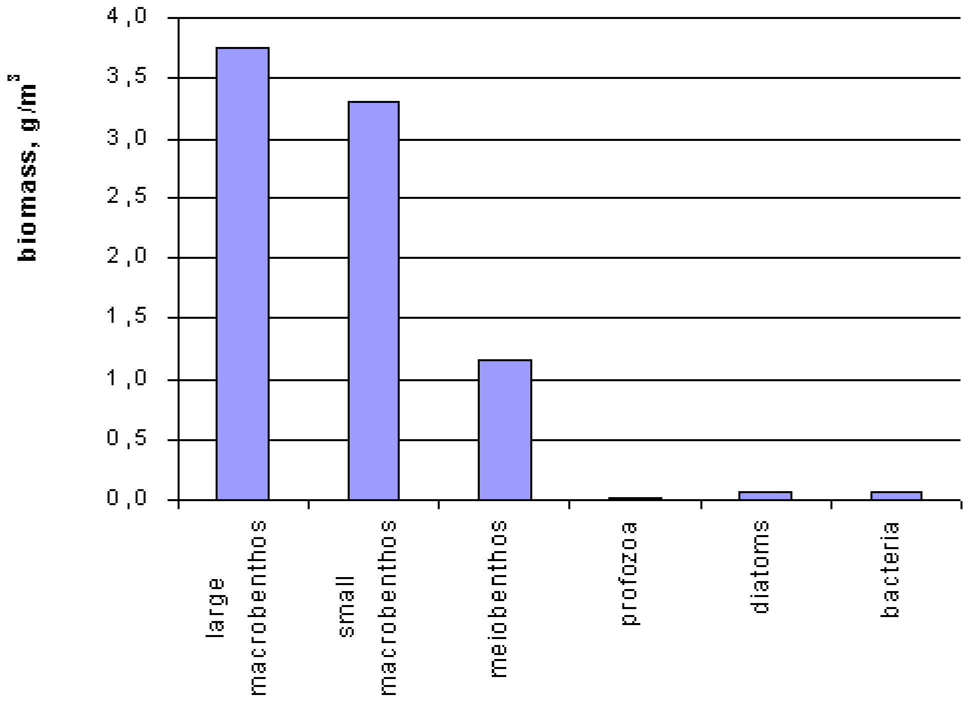

Fig. 2.2. A comparison of the biomass of bacteria with other organisms in the upper layer of bottom sediments [Mare 1942]

2.3. Factors limiting growth of microorganisms in water

The development of microorganisms in water is influenced by a large number of chemical and physical factors which, in various ways, interact or oppose each other (Fig. 2.3). They have an influence on the size and the species composition of the microbial biocenosis as well as on their appearance and life processes.
Within water ecosystems two groups of factors that have a crucial influence on the quantitative and qualitative relationships between microorganisms may be distinguished:

- abiotic factors - light and thermal energy, water reaction, water flow, climate and the compounds dissolved and suspended in water (dead organic matter, non-organic compounds and gasses such as oxygen, carbon dioxide, methane and others).
- biotic factors - all water living organisms such as plants, animals, microorganisms and the relationship between them.

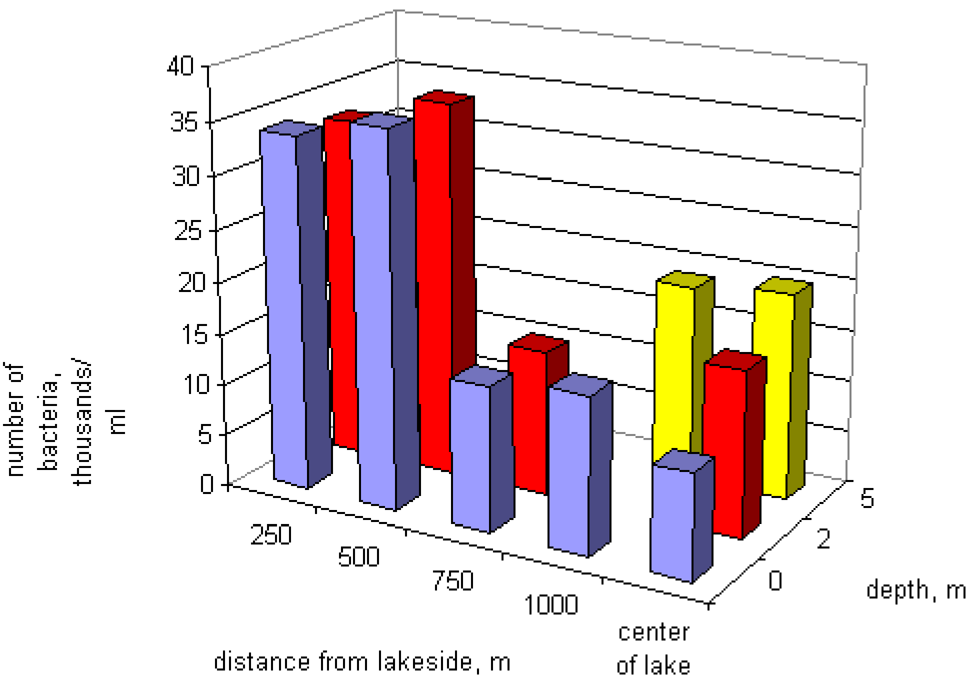

Fig. 2.3. Vertical distribution of bacteria in a lake [Deufel 1969]

2.3.1. Abiotic factors

Light energy
Light plays a major role in the process of photosynthesis. The amount of light penetrating different layers of water strictly depends on the position of the sun, transparency, colour and depth of water. The lesser the incidence angle the smaller the loss of sun rays due to reflection. Depending on the level of insolation and water turbidity, the biologically active sun rays usually penetrate water somewhere between 10-150 m. Undoubtedly, sea waters are clearer and less polluted than inland waters, thus light can penetrate much further down through these waters. Sun rays penetrate sea waters down to about 150 m creating the so called photic zone where photosynthesis takes place.

Due to different light conditions the development of photoautotrophs isn't identical throughout the entire water mass. The indicator of the illumination quantity is often the lower boundary (limit) of algae occurrence – their greatest development takes place at a depth of 0.5-2 m. Most algae possess an ability to change and adapt their colouring to the light conditions.

Light is harmful to those microorganisms which are deprived of any pigments. Both the ultraviolet and the longer wavelength may have a negative effect. For instance, blue light (wave length 366-436 nm) inhibits the process of nitrite oxidation by *Nitrobacter vinogradskyi.* Light also has an influence upon water fungi development. Blue and green rays have a greater impact than red rays.

Temperature
The amount of thermal energy depends, just as in the case of light energy, on the incidence angle (the position of the sun in relation to the water surface). Therefore, it varies with time of day, seasons and latitude.

Lotic waters such as rivers have a steady temperature throughout their mass due to constant mixing by the water flow. However, such a water habitat is characterised by daily temperature fluctuations especially in shallow rivers.

In lentic (stagnant) waters such as lakes, where the water current is very weak or non-existent, the temperature fluctuates during the annual cycle. Lakes, especially deep ones, are characterized by **vertical stratification** (the formation of layers that vary according to their composition and temperature).

Illuminated warm and near-surface waters have a lower density than the dark and cold waters from below. The difference in density prevents mixing of the layers. The warm water layer is called the epilimnion. The cooler layers from below form a thermocline or metalimnion and become cooler with depth. The temperature falls by 1°C with each meter. In the lowest layer **-** hypolimnion – the water is at 4°C and has the highest density.

The thermocline works as a barrier between the epi- and hypolimnion. The upper waters do not mix throughout the year due to their different density. Water is only moved within the epilimnion layer by the wind. The biogenes present near the bottom are not available for the organisms living in the upper layers thus, in late summer; the top layer has a deficit of trophic substances.

In the autumn the surface waters begin to cool down, slowly falling while pushing the warmer waters upwards, which also cool down. As the waters continue to exchange (autumn circulation) and are mixed by the wind they oxygenate and at the same time lose CO_2 by releasing it to the atmosphere especially from the bottom waters.

A slight inversion of temperature occurs in the winter since the water at less than 4°C has a lower density than the 4°C water and it rises towards the surface. Different circulation occurs in the spring as the surface waters warm up. Then, the entire body of water is rich in oxygen and biogenes. The mixing of water also causes organisms to move.

Water movement
Mixing of water is of great importance to both the temperature distribution and for the balance of the chemical composition (gasses, nutrients, substances that equalize the osmotic pressure, water pH etc.). The movement of water is caused by the following:

- variations in density caused by different temperatures and contents of soluble and suspended compounds
- winds
- difference in the levels at the bottom (lotic waters)
- specific hydraulic engineering processes.

Pressure
Pressure is an important ecological factor that strongly influences the life of microorganisms among other things by affecting the activity of the cells enzymatic systems.

In water the hydrostatic pressure gradually increases with depth at 1atm per 10m. Thus, in large oceans and some deep lakes the pressure is quite high - in most seas it's at about 100atm and in some Pacific trenches it may reach even 1100 atm. The group of abyssal microorganisms, which occur at depths of 10,000 m, are called barophilic. They grow and develop not only under great pressures, but also at very low temperature (3 -5°C) but their growth is very slow. Most fresh water and soil bacteria do not develop when the pressure exceeds 200 atm (barophobic microorganisms).

Water pH

An optimal pH for water bacteria is between 6.5-8.5. The pH of most lakes is 7.0, rivers - 7.5, and the surface layer of the seas 8.2. Because of the high content of carbonates and their buffer properties, the pH of water does not usually fluctuate significantly. But when there is a high level of insolation and a rapid growth of photosynthesising organisms the pH may increase rather considerably. Some mineral springs and inland waters with a high content of humus compounds may be acidic. In such conditions the number of acidophilic fungi increases.
Relatively large changes in pH can be observed in eutrophic lakes where the pH varies between 7-10, which has an obvious influence on the populations of bacteria and fungi.

Salinity

Most microorganisms that live in clean rivers and lakes are halophobic and in natural conditions do not live in waters in which the salinity exceeds 10%. There aren't many halophilic organisms which may grow in waters of higher salinity.

Due to the salinity, sea is thought of as a separate (distinct) biotope; the predominant number of bacteria and fungi living in seas are halophilic. Their life processes depend on a specific concentration of NaCl thus, most of the organisms living in such habitats cannot survive anywhere else.

The major mass of salt (99%) is composed of the following elements: Cl, Na, S, Mg, Ca and K.

The concentration of salt in sea water is on average 35‰. The optimal salinity range for most halophilic bacteria and fungi varies between 25-40‰. In the oceans is on average 32-38‰, however in closed seas (salty lakes) the range is much greater. For instance, the Caspian Sea contains a low level of salt (1.1-1.3%), whereas the Dead Sea's salinity ranges up to 28%.

An increase in salinity has an influence on the generation cycle of bacteria and fungi, and on their morphological and physiological properties.

Lakes with a high concentration of salts are extreme biotopes and their biotic groups are low in species variation (the main microorganisms are the bacteria, blue-green algae, flagellates).

Other non-organic substances

The life cycle of water microorganisms is also dependent on non-organic substances other than NaCl, among which phosphorus and nitrogen compounds play a major role.
Besides free nitrogen, many mineral compounds of this element, such as nitrates, nitrites and ammonium salts, occur in surface waters.

Algae and heterotrophic bacteria most often use nitrates and ammonium salts. The maximum amounts of nitrogen which are tolerated by various algae species are different. For instance, diatoms (such as *Asterionella)* may reproduce at high concentrations - even at 100μg N/l, whereas the maximum level for *Pediastrum* algae is only 2μg N/l. It is similar for the bacteria - the maximum amount is different for various species.

The most important element which limits the development of algae is phosphorus. Its content in water is rather low (0.01-0.1 mg P_2O_5/l). Mineral phosphorus occurs in waters in diluted forms (orthophosphate) and in the form of insoluble salts - calcium phosphate, magnesium phosphate etc. Algae may store phosphorus in their cells in amounts exceeding their requirement. The influence of an increasing phosphate concentration by the introduction of pollutants is a reason for water blooming.

In oligotrophic lakes as well as in seas that are nutrient-deficient, it is difficult to detect any presence of ammonium ions, nitrites, nitrates and phosphates since these elements are utilized by phytoplankton immediately after their production. Within the photic zones of many tropical seas the deficiencies in nitrogen and phosphorus compounds last throughout the year, whereas in temperate zones it undergoes seasonal changes.

On the other hand, in the deep waters of some large lakes and seas the accumulation of nitrates and phosphates occurs as a result of heterotrophic microorganism activities. Ammonium ions and nitrites are the energy substrates for the nitrification bacteria whereas, oxygen combined in nitrates may be utilized by a number of denitrifying bacteria to oxidize the organic substances in anaerobic conditions.

Other life essential salts are the compounds of S, Mg, Ca, K, Fe, Si. They are utilized by microorganisms to build cell structures and for the activation of enzymes.

Gases
In water reservoirs, besides salts and organic substances, small quantities of diluted gas can be found. Water possesses an ability to dilute gases but the solubility decreases as the temperature and salinity increase; it is lower in sea waters than in the fresh water basins. It mainly concerns oxygen, carbon dioxide and nitrogen.
The main source of the above gases is the atmosphere from which gases diffuse into the upper layers of water until a state of saturation is obtained. In addition, gases diluted in water and sediments may be created during biochemical processes. In this way oxygen is released by green plants as a result of photosynthesis, CO_2 during respiration, free nitrogen during denitrification, hydrogen sulphide as a result of desulfurication, and hydrocarbons as a result of fermentation processes.

Organic substances
Organic subtances are either secreted by living cells or the products of their autolysis. However, the greatest amounts of organic compounds are introduced into water by sewage. Organic compounds occur in water in the form of solutions or as suspended matter. First of all they serve as food for heterotrophic bacteria and fungi. Microorganisms that often occur on the surface of the suspensions, especially upon the particles of the detritus which absorb the organic substances from water, enjoy favourable feeding conditions.

The development and metabolic changes of microorganisms are influenced, more by the content of readily available organic compounds (such as carbohydrates, organic acids, proteins and lipids) rather than the amount of the organic substances in general. Their depletion from water occurs rather quickly. When there is a lack of organic substances bacteria do not reach their proper size and their cell division is slowed down.

Trophicity of surface waters
Trophicity is the water's abundance of biogenic elements and soluble simple organic compounds. Trophicity determines the primary production rate and the size of the biomass. Major indicators of water trophicity are: phosphorus and nitrogen concentration, chlorophyll concentration, water transparency and oxygen conditions near the bottom.

With reference to the water trophicity, the following kinds of water reservoirs may be distinguished: oligotrophic –low nutrient concentration, mesotrophic – medium nutrient concentration, eutrophic - rich in nutrients (fertile), and hypertrophic - very rich in nutrients (Table 1). The abundance of nutrients in water reservoirs changes with time. This process of water fertility increases from oligothrophic through mesotrophic to eutrophic waters in a process called **eutrophication.** When the above process is moderate and its effects are beneficial, then it is considered to be a fertilization process. However when the process is excessive and its effects aren't beneficial, then it is considered to be biogenic substance pollution. Nutrient deficient low-fertile waters are those which have a low content of phosphorus and nitrogen – it is these two elements that are the most crucial. Therefore the waters contain low numbers of phyto- and zooplankton organisms and they are clean and clear. In waters which contain a low amount of plankton very little dead matter falls to the bottom. Therefore, its decomposition does not deplete the oxygen reserves near the bottom.

Table 1. The number of bacteria in the upper layer of water during summer stagnation [Kuzniecow 1970]

Type of reservoir	Lake	Number of bacteria thousand/ml
Oligotrophic	Bajkal Onega Ladoga	50-200 240-340 100-300
Mezotrophic	Deep (Moscow area) Imoloze (Upper Woloczek) Kolomienskie	1000-1400 420 1100
Eutrophic	White (Kosino, Moscow area) Black (as above) Batorin (Bialerus)	2200 4000 3500-8000

Up to a certain level, the increase in water fertility in turn causes an increase in the number of most organisms in water and consequently an intensification of life manifestations. When the level of nutrients is too high the organic matter produced disturbs the ecosystem's homeostasis. Some enzymes released by bacteria cause decomposition of the other bacteria and algae. Owing to a release of organic substances the plankton microorganisms grow abundantly. The increased use of oxygen causes a deficit of oxygen in deeper waters and consequently, the development of anaerobic microorganisms and the appearance of methane and hydrogen sulphide. Thus, high vitality means increased production in water basins, biomass development of phytoplankton and at the same time lower oxygen concentration in deeper layers.

Water blooming is a consequence of eutrophication and it is caused by the reproduction of algae in the upper layers of water. The above process causes changes in water colour, its turbidity, water quality deteriorates, and toxic compounds are produced.

The process of natural eutrophication proceeds very slowly – it takes up to a few thousand years. Accelerated eutrophication, however, is caused by human activities. As a result, excess amounts of nitrogen and phosphorus get into waters from various sources such as industrial and municipal wastes, as well as fields that have been fertilized with phosphorus and nitrogen fertilizers. Such activities greatly increase the concentration of biogenes creating favourable conditions for algae reproduction. In such polluted waters, faecal and pathogenic bacteria may survive for a longer period of time. Precipitation in highly industrial and polluted areas has also had an influence upon eutrophication.

2.3.2. Biotic factors

Mutual interactions exist between individual members of the biocenosis that inhabit surface waters. As a result, the organisms may support each other (synergism) or inhibit each other (antagonism).

Competition for food

The organisms which most efficiently find and take in food may have an advantage over others. For a given habitat with a typical supply of nutrients, the number of microorganisms quickly increases. However, in many cases, the abundant production of products of metabolism (inhibitors) decreases the number of competitors, sometimes eliminating them entirely. Such situations occur, for instance, when the pH is significantly altered by acidification or alkalisation, and when antibiotic substances are released.

Co-operation

In feeding and growth processes, co-operation between the microorganisms is often observed. It allows quicker development of mixed microorganisms cultures. Biodegradation is a multistage process when consecutive reactions are conducted by different specialized microorganisms. The process prevents the accumulation of the metabolism by-products. Owing to this co-operation, the biodegradation of persistent organic compounds (ligninocellulose) becomes possible.

Predation

Bacteria and fungi are food for lower animals. This is why in some water reservoirs their numbers may vary a lot. Most protozoa feed on bacteria. It has been confirmed that their biomass increases along with the increase in bacterial numbers. Numerous multi-cellular organisms also utilize bacteria as their food. These mainly include filtrating animals such as sponges. In bottom sediments many animals feed upon fungi. Blue-green algae which are a part of the benthos are often eaten by turbellarians, nematodes, crustaceans and insect larva. Blue-green algae are eaten by zooplankton, without the latter water blooming and release of toxic substances would occur.

Parasitism

Water microorganisms are attacked and destroyed by viruses, bacteria and fungi.
The presence of bacteriophages has been affirmed in inland and sea waters. They are especially numerous in sewage and are probably the reason for a quick depletion of bacteria in rivers, lakes and in inshore waters that were polluted with sewage.

Another reason for the limitation of bacterial numbers is the presence of the Vibrio bacteria which belong to *Bdellovibrio* genera and lead a parasitic existence. They attach themselves to host cells and reproduce utilizing their energy and consequently digest the cells content. After lysing the host's cell wall, they free themselves and infect further bacteria.

2.4. Characterisation of water microorganisms

2.4.1. Bacteria

In terms of morphology, most water bacteria resemble soil bacteria - their cells are round, cylindrical or screw like. There are also thread-like and stem-like shapes. The threads may be or not be branched, single or in groups. Various water bacteria may create clusters made up of various numbers of cells in the following shapes: spherical, star-shaped, lamellar, and filiform.

Most water bacteria are active and mobile using cilia or flagella (e.g. *Vibrio, Pseudomonas).* Bacteria in water may swim slowly (plankton) or occupy a fixed substrate. Oligotrophic water bacteria in clean waters occur as microforms with cells smaller then 1μm, usually 0.4μm.

Water oligotrophs rarely multiply, their generation cycle lasts from a few dozen to two hundred hours. Polluted waters are predominantly occupied by bacteria of the Gram-negative rods group. The ratio of rods to cocci is about 90:1. Clean waters (rivers and streams) contain a sparse microflora and the ratio of rods to cocci is 1:1,5, which indicates the dominance of cocci.

The number of bacteria in water depends mainly on the organic matter content. In clean waters they occur in low numbers whereas polluted waters contain up to several million cells per 1ml of water (Table 2).

Table 2. Estimates of bacterial numbers in lotic waters with various pollution levels [Cabejszek 1957]

Pollution level	Number of bacteria upon a simple agar at 37°C after 24h in 1ml	Number of bacteria upon gelatin at 20°C after 48h in 1ml	Coli titre upon Eijkman's nutrient medium at 37°C after 48 h
Unpolluted water	up to 200 cells	up to 300 cells	more than 1
Slightly polluted water	up to 1000 cells	up to 5000 cells	1-0.1
Significantly polluted water	1000-5000 cells	5000-10 000 cells	0.1-0.01
Highly polluted water	more than 5000 cells	more than 10 000 cells	less than 0.01

Bacteria that occur in water habitats may be divided into the following:
- autochthonous (native) constantly occupying water habitats
- allochthonous (foreign) finding their way from the soil or the air as well as microorganisms that get into the water basins along with municipal and industrial sewage.

A. Autochthonous bacteria

We can distinguish photoautotrophs, chemoautotrophs and chemoorganoautotrophs

- photosynthesizing bacteria (photoautotrophs)

Purple and green bacteria are among the photosynthesizing autotrophs. Due to their metabolism these bacteria can be divided into the following groups:
- Filiform green bacteria *(Chloroflexaceae*),
- Sulfuric green bacteria (C*hlorobiaceae*),
- Sulfuric purple bacteria (*Chromatiaceae* and *Ectothiorhodaceae*),
- Non-sulfuric purple bacteria (*Rhodospirillaceae*),
- Heliobacteria (*Heliobacteriaceae*).

The photosynthesis of bacteria is carried out slightly differently to that of plants. Most importantly, it is an oxygen-free process which requires the presence of reduced mineral compounds and it is not accompanied by a release of oxygen but by a production of oxidized non-organic or organic compounds. The assimilating pigments of bacteria are categorized by the ability to absorb infrared light that is not absorbed by green plants. The photosynthesis in surface waters is conducted mainly by algae and plants and the role of the bacterial photosynthesis is less important.

- chemosynthesizing bacteria (chemoautotrophs)
Chemoautotrophs get energy from the oxidation processes of non-organic compounds. Depending on the nature of the oxidized substrate the following can be distinguished: nitrifying, ferruginous, sulfuric and hydrogen bacteria.

- The role of the **nitrifying bacteria** in surface waters is the oxidation of ammonia and nitrite to nitrate. In greater concentrations the above compounds may be harmful to water organisms as well as to humans (in cases when such water is utilized for water supply systems). Moreover, the production of nitrate is a fundamental process that supplies water plants with a source of nitrogen.
- **Ferruginous bacteria** grow in waters when the content of bivalent iron ranges between 0.15-8.5mg/dm^3. Their negative influence includes corrosion and fouling of plumbing, sewage systems and different metal constructions. The most common ferruginous bacteria are the *Leptothrix ochracea and Crenothrix polyspora* and they belong to the filamentous bacteria which are categorized by the fact that the single cells form thread-like forms surrounded by a gelatinous sheath of various thickness. Stored ferruginous substances in cells change the coloration of cells threads into a yellow or dark-brown shade. The ferruginous bacteria are very common in fresh bodies of water. Especially in waters from wells and springs where it is possible to observe their clusters with the naked eye. Moreover, they occur abundantly in muddy streams, marshes and ponds.
- **Sulfuric bacteria** occur mainly in waters containing hydrogen sulfide which is toxic for most microorganisms, whereas for this group it is one of the crucial compounds for survival. These bacteria can be found in mineral springs that contain hydrogen sulfide of geological origin as well as in highly polluted waters where it is produced as a result of oxygen-free protein decomposition or desulfurication processes. The typical representatives of the sulfuric bacteria are: bacteria that move in sliding motions *Beggiatoa alba* and fixed to the bottom *Thiothrix nivea.* The forms of individual sulfuric bacteria are:
    - *Thiobacillus thioparus -* stores sulfur derived from oxidation of thiosulfate
    - *Thiobacillus thiooxidans -* grows in acidic habitats of pH 1.0-4.0
    - *Thiobacillus ferroxidans -* besides thiosulfates and tetrationans it possesses the ability to decompose ferruginous salts.
    - *Thiobacillus denitrificans -* is a relative anaerobe and it has an ability to utilize nitrates as the electron acceptor during the oxidation of hydrogen sulfide. In aerobic conditions the above function is performed by the oxygen.
- **Hydrogen bacteria** posses an ability to oxidize hydrogen using oxygen as a final acceptor of electrons. Most often they feed heterotrophically and switch to autotrophic feeding when hydrogen is present in the habitat. The most widespread species belong to the genus *Hydrogenomonas. Micrococcus denitrificans* belongs to a group of the hydrogen bacteria and they conduct the oxidation of hydrogen while simultaneously reducing nitrate down to molecular nitrogen. *Desulfovibrio desulfuricans* also oxidizes hydrogen while reducing sulphate down to hydrogen sulphide.

- heterotrophic bacteria (chemoorganotrophs)
A predominant part of autochthonous bacteria which occur in water basins are the chemoorganotrophic bacteria which belong to a group of saprophytes that feed upon dead plant and animal organic matter.

Typical bacteria plankton that occupy an entire water mass are the cilliated Gram-negative rods and they represent the following genera: *Pseudomonas, Achromobacter,* *Alcaligenes, Vibro* and *Aeromonas,* as well as the Gram-positive cocci that belong to the *Micrococcus* genus, treponema and spiral bacteria of the *Spirillum* genus.

The underwater parts of higher plants and the underwater fixed particles are colonized by numerous stem-like bacteria (e.g. *Caulobacter)*, sheathed, filiform, and gemmating bacteria (*e.g. Hyphomicrobium)*, which are one of the microorganisms forming the periphyton. Organisms which usually grow in bottom sediments are oxygen-free putrefactive bacteria, then oxygen-free cellulolytic bacteria and finally oxygen-free chemoorganotrophs such as *Desulfovibrio* genus that reduce sulfate down to the hydrogen sulphide. In addition there are some less numerous oxygen-free methane-generating bacteria which reduce organic compounds down to methane

B. Allochthonous bacteria

Waters of high fertility and also highly polluted surface waters are abundant in saprophytes and parasitic bacteria from among which, the following are predominant: Gram-negative intestinal rods of *Escherichia coli* as well as the *Proteus* genus, *Klebsiella and Enterobacter,* and also rods of *Pseudomonas aeruginosa* and of the *Arthrobacter* genus. Moreover, Gram-positive rods (bacilli) of the *Bacillus, Corynebacterium* and *Clostridium* genera, which are washed out from the soil and get into the bodies of water during heavy rainfalls, also belong to the allochthonous bacteria.

Municipal wastes are the main source of pathogenic bacteria. Moreover, during the infiltration processes and surface run-offs, soil bacteria find their way into the waters as well. The role of air in water contamination is significant in densely populated areas of cities and industrial regions.

2.4.2. Water fungi

In contrast to bacteria which grow best in waters of pH between 6-8, fungi occur only in waters below pH 6.0. Usually fungi occur in shallow waters, right on or just below the surface, which is closely connected to the fact that the organisms require significant amounts of oxygen (Table 3).

The predominant fungi in water environments are represented by mold fungi which belong to the *Oomycota* class (*Leptomitus, Phytophthora*) and to the class of *Zygomycota* (*Mucor* and *Rhizopus).* Relatively frequently fungi belonging to *Ascomycota* as well as the *Deuteromycota* are found in surface waters.

Almost all fungi are heterotrophs that decompose organic matter; waters are occupied by both saprophytes and parasites which colonize water plants and animals. They have more diverse shapes than bacteria and they differentiate into larger cells and more complicated structures. In addition to unicellular ones there are also multi-cellular fungi with large mycelium.
Fungi usually do not occur in clean waters. They grow in abundance on the bottom of waters polluted by sewage (e.g. *Leptomitus lacteus*).

Table 3. Number of yeast in the lower Łaba in 1956/1957
[Pheinheimer 1965]

Content	course of Łaba (in km)						
	474	510	550	586	610	626	666
	The amount of yeast in 1 cm^3 of water						
Summer average	42	35	20	16	14		
Winter average	70	65	58	60	46		

2.4.3. Blue-green algae

Blue-green algae are a group of organisms previously considered to be algae. Currently they are classified to the *Procaryota* kingdom and the sub-kingdom of *Eubacteria.* There are unicellular, colonial (loose cells connected with a single mucus envelope) and filamentous forms (Fig. 2.4).

The prokaryotic organisms contain a nucleoid instead of an isolated nucleus. In contrast to other bacteria they are capable of conducting oxygen photosynthesis. They contain chlorophyll and sometimes disguise it in other photosynthesizing pigments: ficocyanine and alloficocyanine.

Characteristically the blue-green colouring of blue-green algae comes from the combination of chlorophyll and ficocyanine. Blue-green algae reproduce mainly through proliferation by cell fission. Their characteristic trait is that they possess gaseous vacuoles which allow movement in water to places of better illumination. Some (*Anabaena)* are capable of binding atmospheric nitrogen in structures called heterocysts. Due to their resistance to extreme environmental conditions they are ubiquitous. They can be found in deserts and in hot springs. Blue-green algae can cause blooming in lakes and other water reservoirs. Some of them produce toxic metabolites.

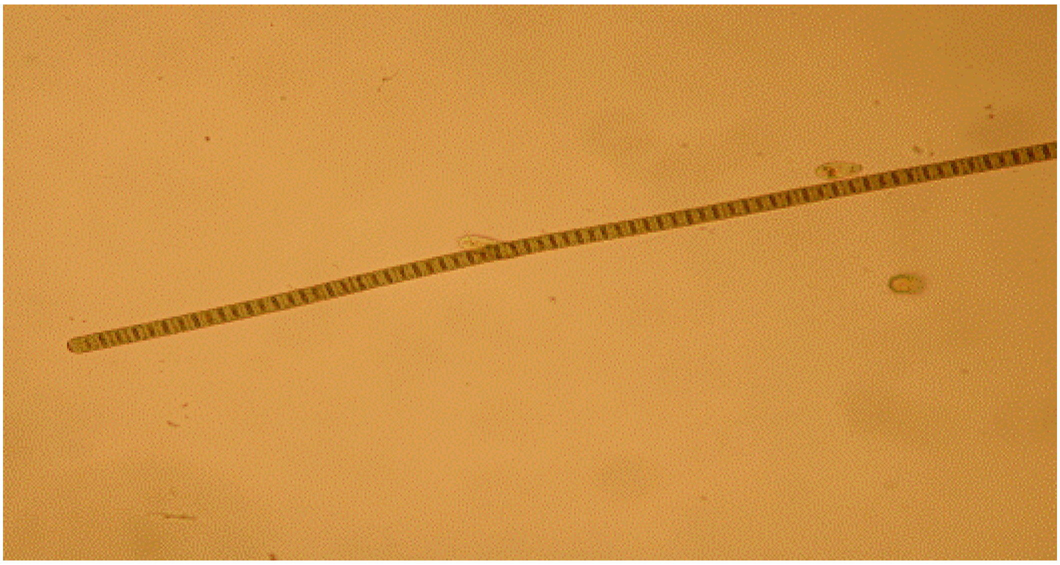

Fig. 2.4. Blue-green algae – *Oscillatoria sp*.

2.4.4. Algae

Algae are the simplest autotrophic eukaryotes that incorporate over 20 thousand species. Algae occur in fresh and sea waters. They are important producers of organic matter and oxygen. Algae live in the form of single cells or they create multicellular body of various shapes called thallus (threads, spheres, multilayer clusters).
The composition of algae community changes significantly with respect to quality and quantity, depending on the content of the mineral salts in any given reservoir as well as on the characteristics of the substances that make up the main pollutant.

The following are the characteristic algae that occur in oligotrophic waters: diatoms of the following genera: *Asterionella*, *Tabellaria, Melosira* and some other algae (*Dinobrion*). In eutrophic waters the content of algae is completely different. Most of all, such waters contain only a vestigial number of diatoms, and instead of them the algae from the *Dinophyta* class as well as the *Spirogyra* genus appear.

Algae are subdivided into the following classes:

- *Chlorophyta* - green algae (Fig. 2.5-6) that contain chlorophyll *a* and *b* types, a cellulosic cell wall and starch as reserve material. They have a diverse constitution, both unicellular and multi-cellular forms exist usually as thread-like structures. Cells may be motile, (then they are equipped with flagella), or non-motile. Chromatophors of various shapes have a green coloration. They reproduce vegetatively or sexually. Vegetative reproduction consists of the division of cells and the fragmentation of thread-like forms.

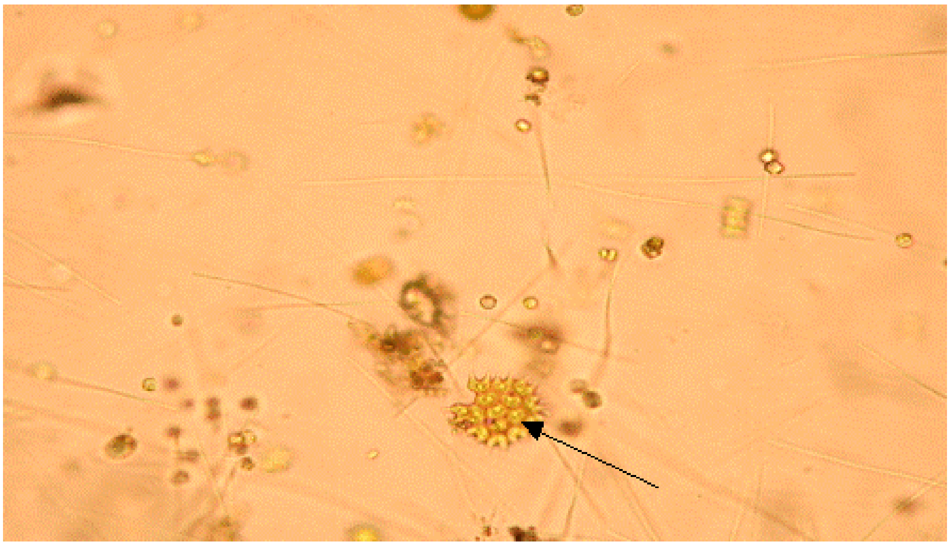

Fig. 2.5. Algae living in Odra river (Poland) – *Pediastrum sp.(Chlorophyta)* (arrow)

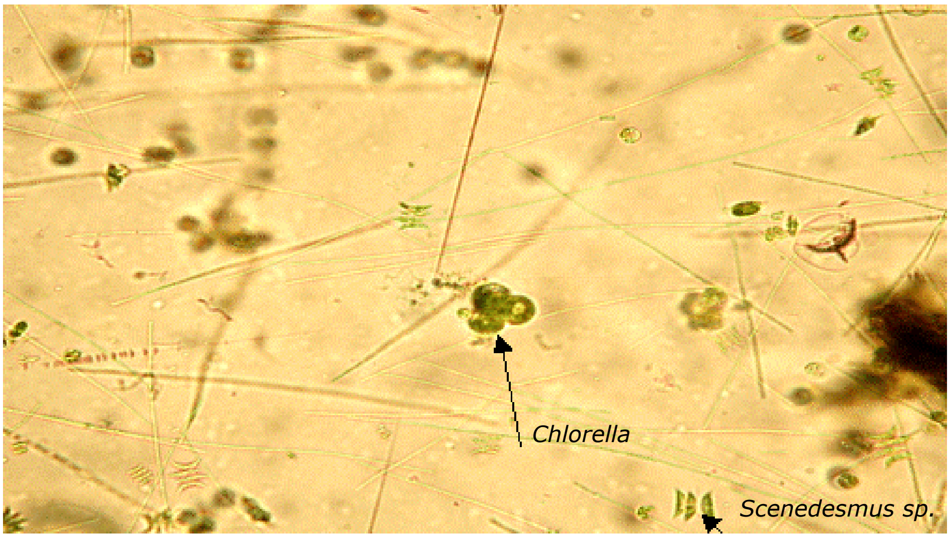

Fig. 2.6. Algae living in Odra river (Poland) – *Chlorella sp.* (left arrow) *and Scenedesmus sp.(Chlorophyta)*

• *Chrysophyta*(fig. 2.7-2.9) - this group involves diatoms important for the water environment. They are common algae and occur in fresh and sea waters, bottom sediments and soil. *Chrysophyta* contain *a* and *c* types of chlorophyll. Their cell wall is enriched in silica. They produce lipids as reserve material.

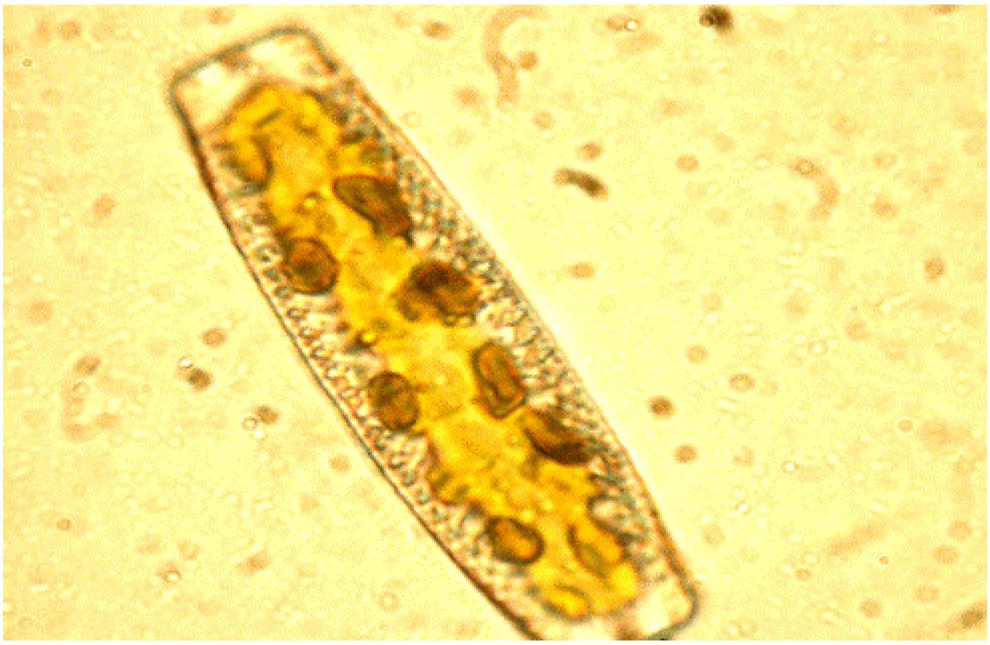

Fig. 2.7. Diatom - *Epithemia* sp.

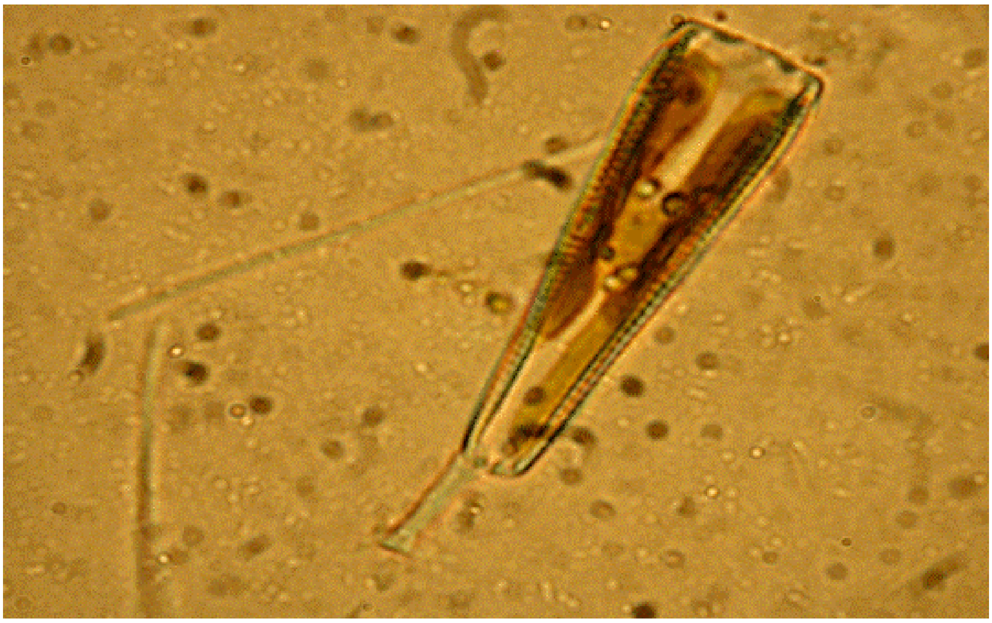

Fig. 2.8. Diatom *Gomphonema constrictum*

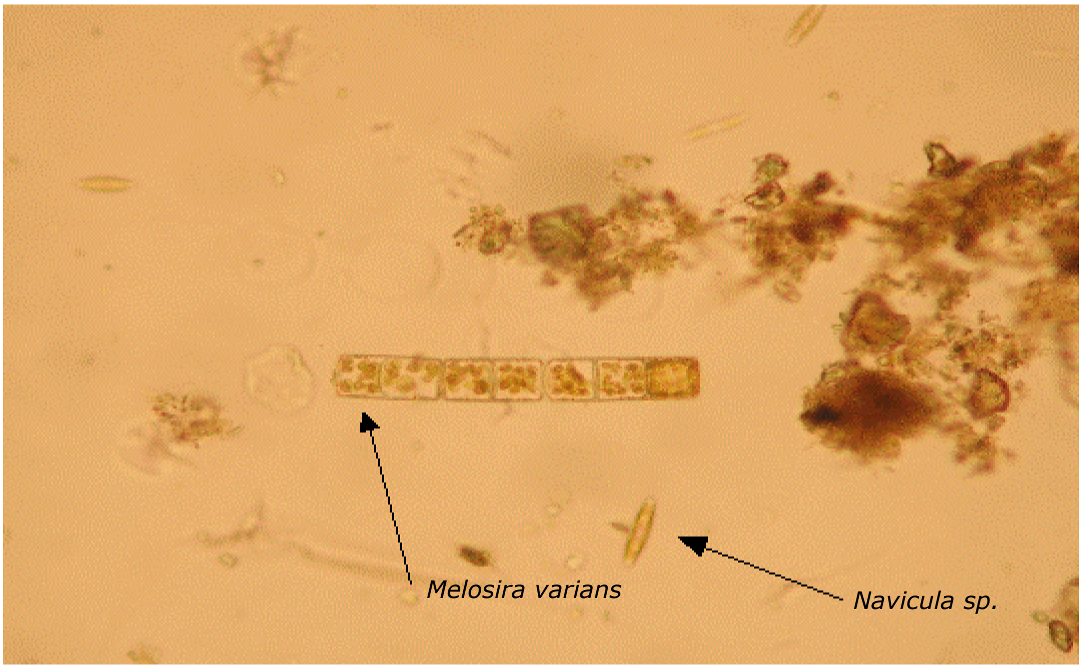

Fig. 2.9. Diatoms occur in Odra River: *Melosira varians* (left arrow)*, Navicula* sp.

• *Euglenophyta* (Fig. 2.10-11). Euglenoids usually have an elongated shape. Their cells are equipped with flagella that allow movement in water or they move by crawling along the bottom. The cells are surrounded by a soft envelope called the pellicle. Chromatophores contain chlorophyll, carotenes and xanthophylls. Within the cell there is a clearly visible nucleus and the eyespot called stigma, sensitive to a light stimulus. Euglenoid cells create cysts which make survival in unfavourable conditions possible. They grow in waters containing high levels of organic compounds. There are also parasitic forms.

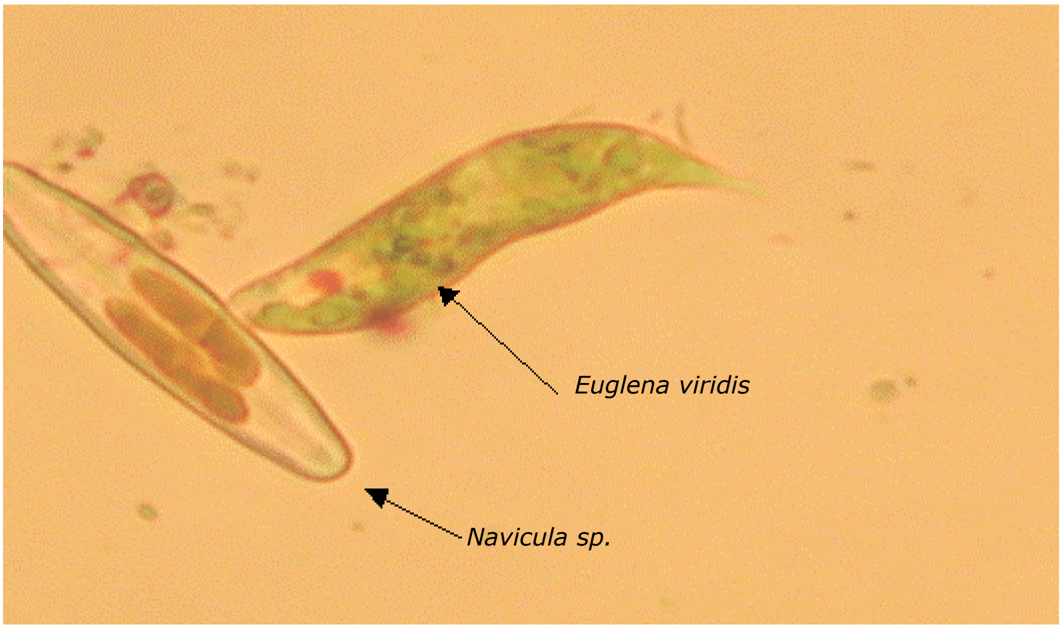

Fig. 2.10. *Navicula* sp. (left arrow) and *Euglena viridis*

Fig. 2.11. Euglenophyta - *Euglena acus*

- *Pyrrophyta o*ccur usually individually. Some cells are surrounded by a cellulose wall whereas others are deprived of any cell walls. They usually possess two flagella that allow their movement. Within the protoplasm there is an isolated cell nucleus and yellow-green or yellow-brown chromatophores. They reproduce by division and some have been observed to reproduce sexually. *Pyrrophyta* occur in slightly salty or sea waters and only selected species live in fresh waters. In lakes there is *Ceratium hirundinella,* a species that sometimes appears in large masses.
- *Rhodophyta* contain chlorophyll type *a* and *b* as well as other pigments such as carotene, xanthophylls and phycobilin pigments: phycoerythrin, phycocyanin.They store starch as a storage product. Their cell wall has two layers: the inner one is made of cellulose whereas the outer one is made of pectin. They reproduce asexually through fragmentation of the thallus and sexually through oogamy.
- *Phaeophyta* - brown algae contain chlorophyll a and c as well as carotenoids (fucoxanthin). Their reserve material is laminarin (β-1, 3-glycan) and chrisolaminarin*,* mannitol and lipids. Their cell wall is also a by-layer: the inner wall is made of cellulose whereas the outer wall is made of pectin. Brown algae are multi-cellular organisms that posses the highest level of specialization of the thallus of all algae and high anatomic and morphological variation. They reproduce by zoospores and also sexually by gametes.

2.4.5. Water protozoa

Protozoa live in all types of waters, from small puddles, to inland waters, to the seas. They feed heterotrophically absorbing the dissolved organic compounds or feeding upon bacteria. They are most numerous in highly polluted waters and are the element of activated sludge. When the pollution level is not too high ciliates become predominant, and that concerns both the free-swimming ones (e.g. *Paramecium*) and the settled ones (e.g. *Vorticella).*

Protozoa can be subdivided into four classes:

- *Flagellata* – flagellates (Fig. 2.12). These move utilizing long flagella. They feed heterotrophically and occur in polluted waters or in inefficiently functioning activated sludge. Besides dissolved substances, they may also absorb bacteria or unicellular algae. Flagellates live individually or in colonies.

There are parasitic forms among them too. This is exemplified by a human parasite *Giardia lamblia* and the *Trypanosoma gambiense* which is transferred on to humans by the *Tsetse* fly causing African sleeping-sickness and neurological disturbances.

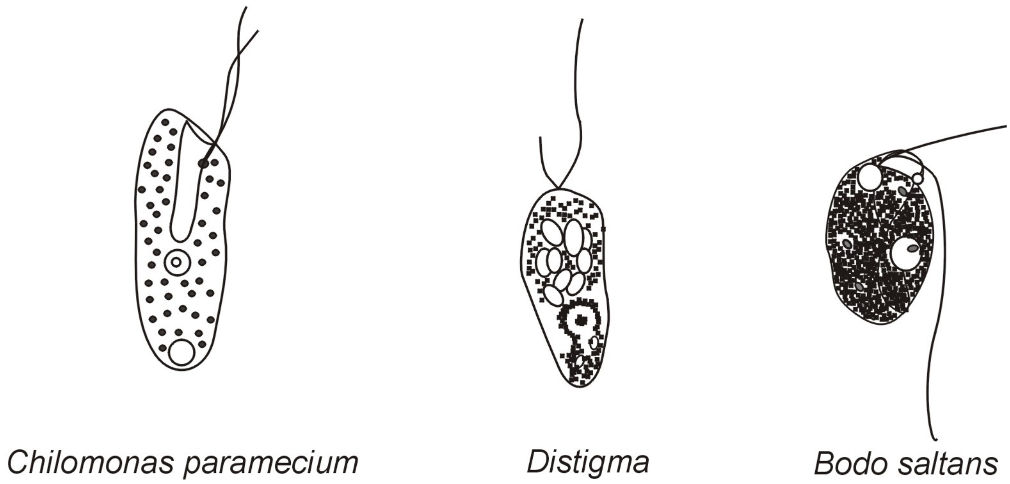

Fig. 2.12. Typical water flagellates

- *Rhizopoda*- amoebae (fig.13) the cells move around utilizing the plasmatic pseudopodia which are used for locomotion and for capture of food. Some amoebae have a changeable shape, others however, have a constant shape as they are equipped with a mini-skeleton or an outer shell. Some amoebae lead a parasitic life (*Entamoeba histolytica).*

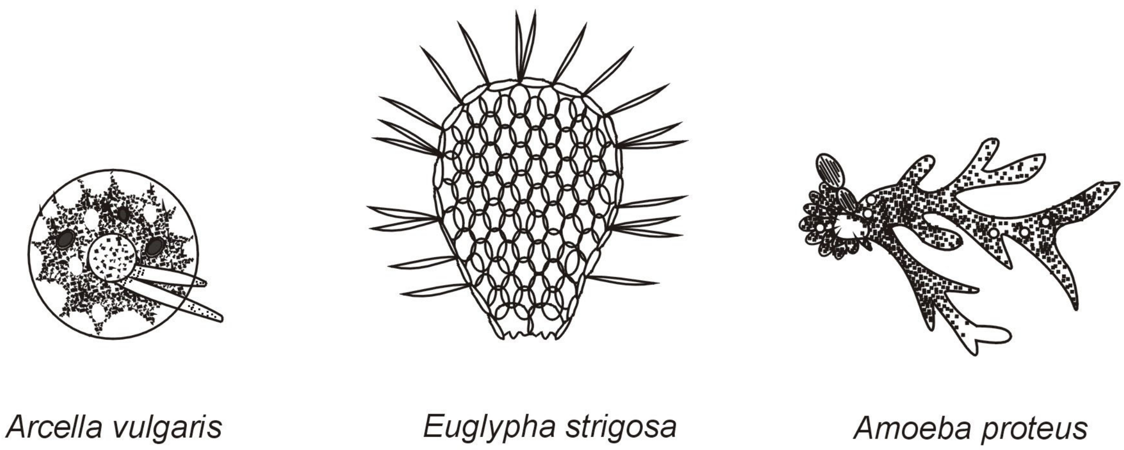

Fig. 2.13. Typical water amoebae

- *Ciliata* – ciliates (fig.14-17). Most of the representatives lead a free-swimming life style (*Paramaecium, Euplotes),* others crawl or are attached to the bottom. They feed upon bacteria, algae and organic substances. Ciliates occur in large numbers in polluted waters and in activated sludge. Some, such as *Balantidum coli* which causes dysentery, are parasites of animals and humans.

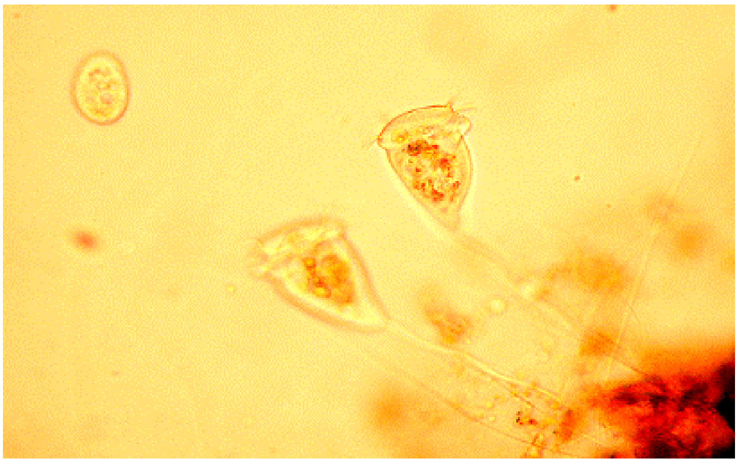

Fig. 2.14. Protozoa – Ciliata *Vorticella convallaria*

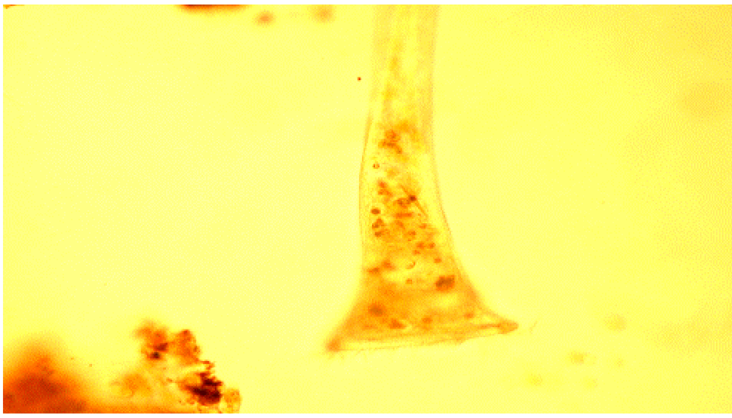

Fig. 2.15. Protozoa – Ciliata *Stentor* sp.

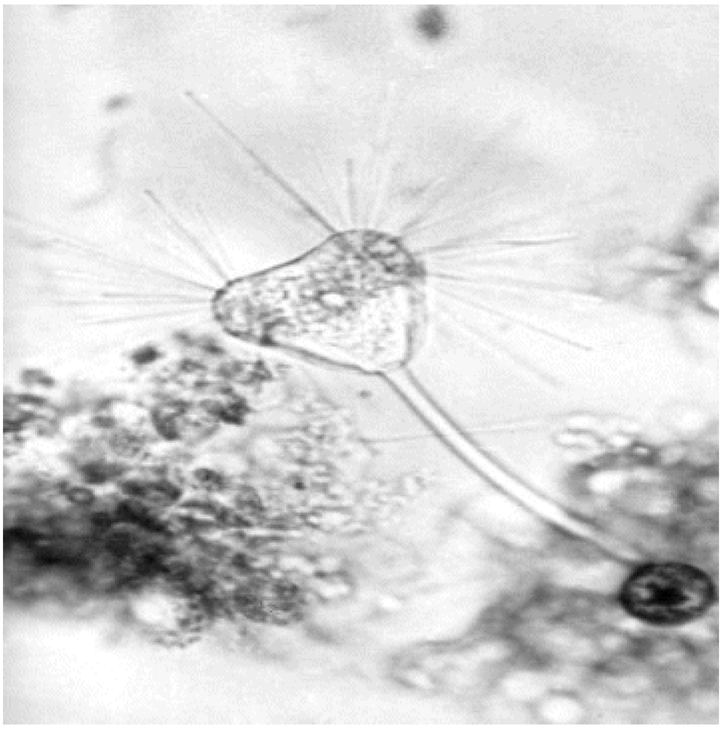

Fig. 2.16. Ciliata – Suctoria

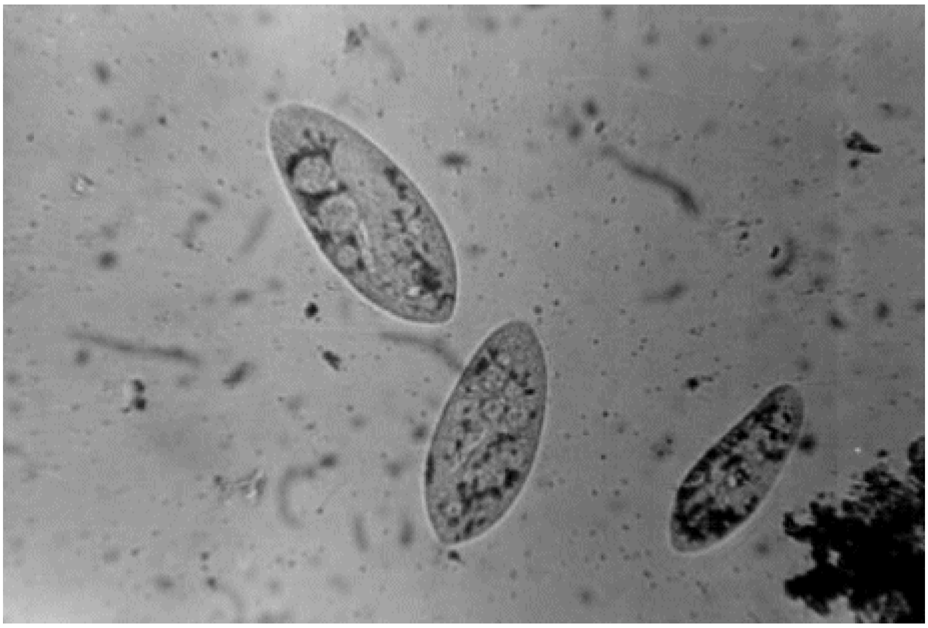

Fig. 2.17. Free-swimming ciliates – *Paramecium* sp.

- *Sporozoa.* Only parasites belong to this class and representatives are *Cryptosporidium parvum* causing intestinal diseases and *Plasmodium malariae* causing the malaria. The second parasite attacks the red blood cells. This pathogen is carried by the *Anopheles* mosquito.

2.5. Polluted water organisms

2.5.1. Sources and types of pollutants

Waters become polluted as a result of domestic and industrial sewage disposal into surface waters, which contain huge amounts of various compounds that affect the biocenosis of water reservoirs. Besides sewage, pollution is also caused by rain run-offs which wash away different fertilizers and crop protection products. Moreover, the pollutants also transfer into waters from the surrounding air. This usually results from industrial dust which falls directly into the water or is washed away from the ground surface by rain. Important gases are: sulphur dioxide, nitrogen oxides, carbon oxides and dioxides which get into the waters mainly in highly industrial areas.

Some of the above compounds undergo microbiological decomposition relatively easily, becoming food for heterotrophic microorganisms, others are resistant to such decomposition and are harmful or toxic to microorganisms. Examples of these are the following: cyclic compounds, engine oil, lubricants, chlorinated hydrocarbons, pesticides, and among the mineral pollutants – heavy metal salts.

2.5.2. Self-purification of surface waters

Self-purification encompasses complex co-operation between physical and biochemical factors such as: sedimentation (settling), oxidation, an exchange of volatile substances between the atmosphere and water, and the release of gaseous products of metabolism into the atmosphere. However, the critical role is played by the biological factors.
A wide range of microorganisms and higher organisms participate in self-purification processes.

Bacteria and fungi are the most crucial as they are capable of mineralising various mineral components. Proteins, simple and complex sugars, fats, cellulose, lignin, wax and others undergo degradation during the process of self-purification. As a result of mineralization the following compounds are created: H_2O, CO_2, NO_3^-, SO_4^{2-}, PO_4^{3-}, and other simple compounds. With the progression of self-purification the populations of microorganisms that act in the environment change (Fig. 2.18).

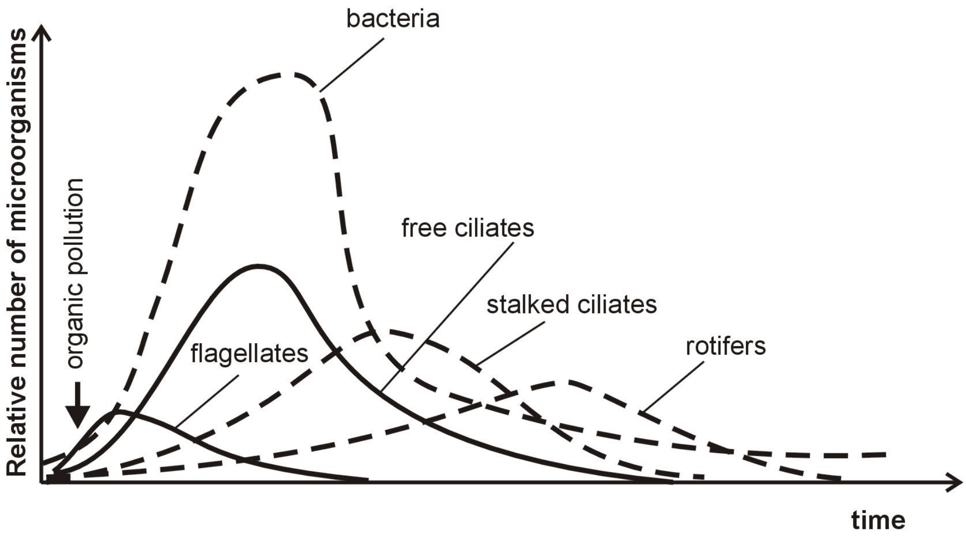

Fig. 2.18. Succession of microorganisms during the self-purification process

The self-purification process utilizes large amounts of oxygen during the biochemical processes. The amount of oxygen that is used up in any specified time by water microorganisms is called the **biochemical oxygen demand, BOD.** By analysing the BOD, it is possible to determine the concentration of the organic compounds dissolved in water which are susceptible to biological oxidation. The discharge of impurities into the water reservoir creates a sudden change in chemical, biological and physical conditions. Simultaneously, right below the area of the discharge, the process of self-purification begins. The process leads to the formation of zones containing characteristically gradually decreasing levels of pollution.

2.5.3. Saprobic zones. Self purification of lotic waters.

Zones of various levels of organic pollution are called saprobic. In particular zones the content of biocenoses is different and altered to fit the existing conditions. Species which clearly dominate other species and have adapted to the existing conditions are found there.

Zones differ in the dynamics of dissimilation processes, the intensity of oxygen intake, and the appearance of the water. There are 3 different saprobic zones: poli-, mezo- and oligosaprobic.

Polisaprobic zone (Fig. 2.19) - is the zone of the highest concentration of pollutants with cloudy, dirty-grey, fetid odorous waters. The high concentration of various organic compounds ensures development of selected heterotrophic microflora which, while conducting biodegradation, use up large amounts of oxygen leading to its deficit. In anaerobic conditions the following gasses are formed: H_2S, NH_3, CH_4, N_2 and others. There is a lack of green plants in this zone. Among the organisms which are capable of surviving in such conditions, the dominant ones are *Zooglea ramigera* and *Sphaerotilus natans* bacteria. There are also sulphur bacteria (in the presence of hydrogen sulphide) especially of the *Beggiatoa* and *Thiothrix* genera; protozoa are also numerous. Reducers (decomposers) are the most frequently occurring organisms in this zone.

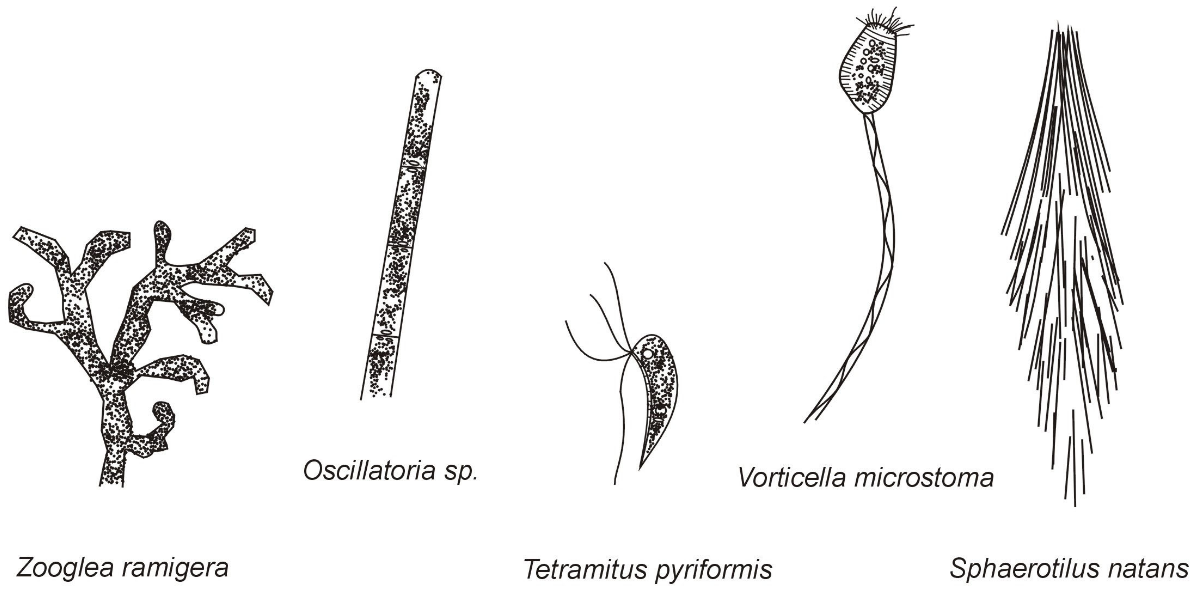

Fig. 2.19. Microorganisms typical for polisaprobic zone

In the mezosaprobic zone there is further intensive breakdown of the organic compounds but the amount of oxygen is sufficient to sustain a full demand. The water becomes clear, often of green coloration due to the abundantly flourishing algae.
The number of reducers decreases. Besides the microorganisms mentioned above, sewage fungi *Leptomitus lacteus*, blue-green algae, sparse diatoms and green algae appear. The mineralization of the organic compounds is finished within the zone, excluding, humus compounds which are difficult to decompose. The mezosaprobic zone is divided into α-and β-zones. The α–mezosaprobic zone is a heterotrophic one in comparison to the β-mezosaprobic zone which is rather autotrophic. The β–mezosaprobic zone is cleaner and higher numbers of algae species occur here (Fig. 2.20-21).

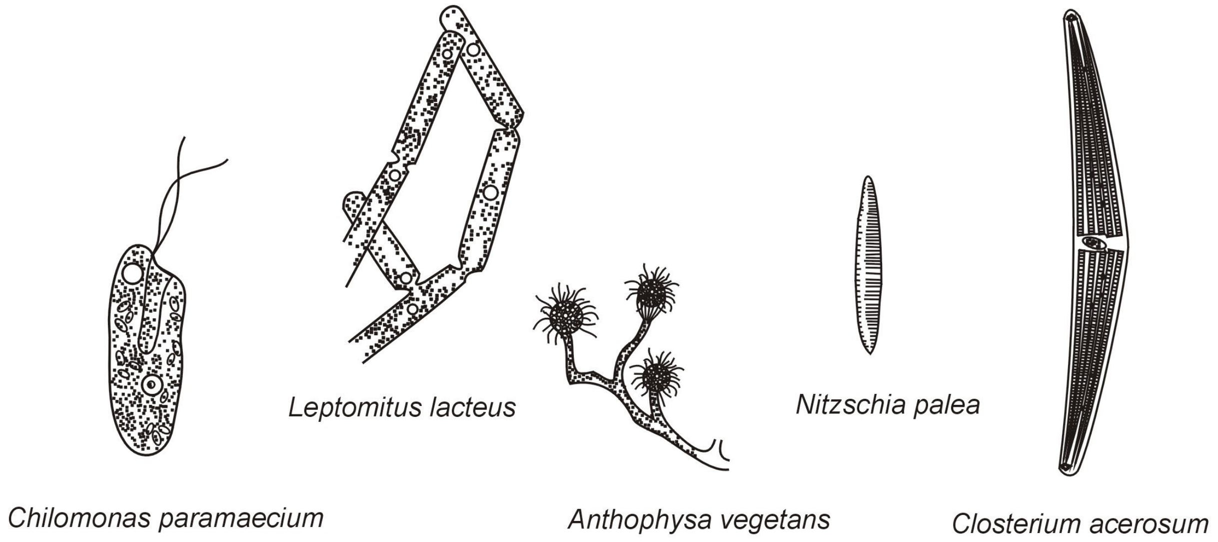

Fig. 2.20. Microorganisms typical for an α-mezosaprobic zone

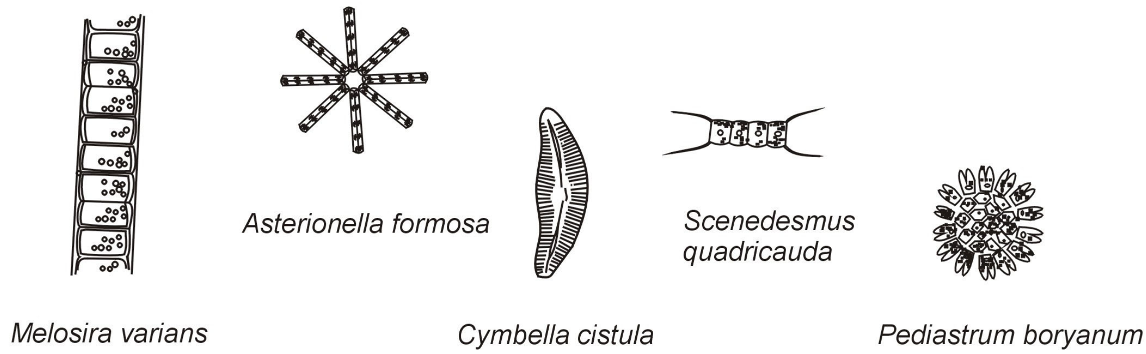

Fig. 2.21. Microorganisms typical for a β-mezosaprobic zone

Oligosaprobic zone is a section where the inflow of impurities ends and water returns to its previous state of natural water. Water is clear, odorless and well oxidized. The zone is mainly inhabited by ferruginous and nitrifying bacteria (the chemosynthesizing bacteria); sparse blue-green algae, many diatoms and green algae, and few protozoa occur in the biocenose (Fig. 2.22).

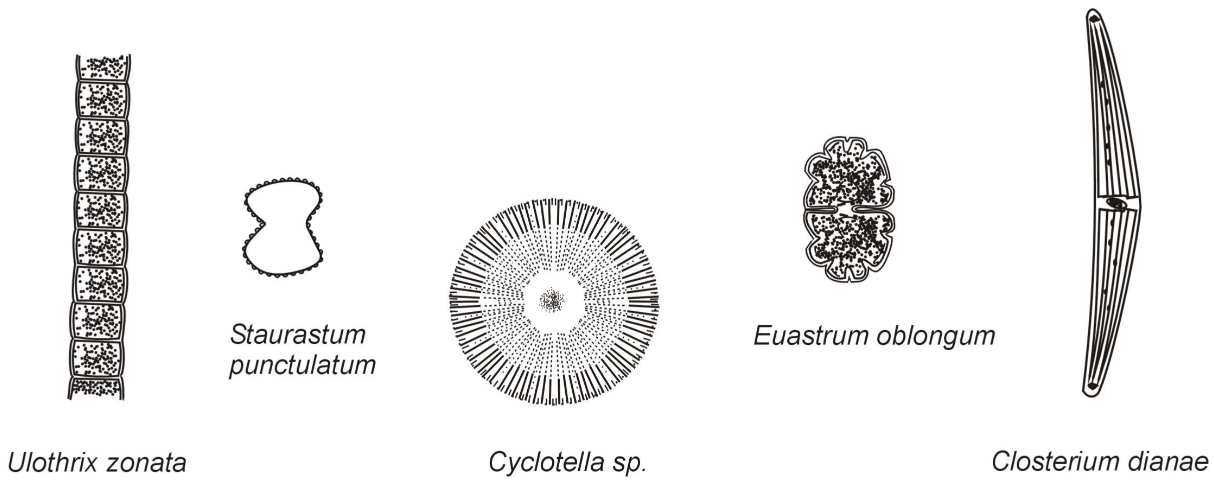

Fig. 2.22. Microorganisms typical for oligosaprobic zone

Self-purification of lentic waters
A self-purification process takes a different course in lentic waters; the saprobic zones do not evolve here even though the purifying mechanism remains the same.
The impurities introduced into water fall down to the bottom (their density is greater than that of water) where they are decomposed.

2.5.4. Microbiological processes within bottom sediments
There are complicated chemical, physical, and biological processes taking place between water and the bottom sediments, which are important for the reservoir as a whole. The water/sediment arrangement, the microorganisms quality-quantity ratio and the direction of bio-chemical changes have a significant influence upon the level of biogenes (nitrogen, phosphorus, sulphur compounds) within the reservoir (its fertilization).

In the bottom sediments, the aerobic decomposition of organic constituents takes place in the upper layers (from a few to several mm) and are a source of soluble mineral salts. Whereas, the anaerobic biodegradation, which takes place below, causes a release of substances often poisonous to the water habitat (e.g. H_2S, CH_4).

Bottom sediments play an important role in lentic water self-purifying process where the organic suspended matter falls to the bottom as a result of lack of water movement. They have a significant influence upon the conversion of the biogenic compounds which affect the quality of water. The most crucial factor regulating the speed of nitrogen and phosphorus penetration (also Fe and Mg) from within the sediments into water is the content of the dissolved oxygen within the layer near the bottom. Active diffusion of phosphates into water begins when the content of the dissolved oxygen falls below 1mg O_2/dm^3. Moreover, the following also have an influence upon the release of phosphates: temperature, organic compound decomposition, water pH, redox potential.

In bottom sediments of various surface water reservoirs, the following microorganisms are the most common: aerobic cellulolytic bacteria (of the *Sporocytophaga, Cytophaga, Pseudomonas, Achromobacter* genera) as well as anaerobes such as the *Clostridium* genus. The latter takes part in the decomposition process of hemicellulose.
In oxygen-free conditions numerous putrefying bacteria (releasing H_2S from proteins), SO_4^{2-} reducing bacteria, denitrifying bacteria (NO_3^- reducing), methane generating (CH_4 releasing) and hydrogen bacteria grow.

Moreover, ammonificating bacteria are abundant in bottom sediments. Nitrifying bacteria usually occur in small numbers in upper layers of sediments as they are obligate aerobes. The presence of CH_4 oxidizing aerobic bacteria in bottom sediments also depends upon the concentration of oxygen and iron.

2.5.5. Water transmitted pathogenic microorganisms

Bacteria (see table 4)
The group of **obligate pathogenic** bacteria, which occur in polluted surface waters, contain rods causing typhoid fever (*Salmonella typhi),* as well as other Gram-negative bacteria of the *Salmonella* genus, which are the cause of various infections of the digestive tract.

Bacterial dysentery caused by Gram-negative rods of the *Shigella* genus are not as common as the above. In surface waters of tropical countries, bacteria of the *Vibrio cholerae* genus (cholera) frequently occur.

Moreover, *Mycobacterium tuberculosis* causing tuberculosis and treponema of the *Leptospira* can be also found in polluted waters. The latter bacteria cause bacterial jaundice.

Beside the obligate pathogenic bacteria, there are also numerous Gram-negative bacteria in surface waters which are described as opportunistic microorganisms (facultatively, pathogenic). These belong to the *Pseudomonas, Aeromonas, Klebsiella, Flavobacterium, Enterobacter, Citrobacter, Serratia, Acinetobacter, Proteus and Providencia* genera. All of the rods are part of the usual flora of the intestine and are not typically pathogenic for as long as they occur in human or animal digestive tracts. In some cases though, these bacteria find their way into other organs becoming a potential cause of different illnesses such as inflammation of urinary and respiratory systems and also sepsis which is a general infection of all internal organs.

Viruses (see table 5)
Besides pathogenic bacteria of surface waters, into which municipal and industrial sewage is disposed, the waters also contain significant amounts of other pathogenic microorganisms such as the *polio* virus. They are responsible for causing the Heine-Medina disease (polio). Enteroviruses, which cause intestinal infections, occur even in slightly polluted rivers.

Protozoa (see table 6)
Infections of the digestive tract caused by protozoa may come from contaminated water. Most parasitic protozoa produce cysts which are able to survive inside their host in unfavourable conditions. When the conditions improve, cysts transform into so called trophozoits, the vegetative form occurring in humans.

Parasitic fungi
In polluted surface waters parasitic fungi can also occur, for example *Microsporum* sp., *Trichophyton* sp. and *Epidermophyton* sp. They are dermatophytes causing ringworm and other cutaneous infections.

Parasitic worms (see table 7)
Human parasites are not usually included in the scope of microbiological research however, along with other pathogens (viruses, bacteria, protozoa) they pose a serious threat to human health. They occur in sewage and may find their way into waters from soils as a result of infiltration and surface run-offs. The infectious forms of the parasitic worms are their eggs. The eggs are excreted in great numbers outside the hosts' body along with faeces and spread through sewage, soil or food. The worms' eggs are very resistant to external factors and thus are difficult to eliminate from sewage by chlorination.

Table 4. Waterborne bacterial infections

Disease type	**Species or genera of bacteria**
Typhoid	*Salmonella typhi*
Paratyphoid	*Salmonella paratyphi*
Animal salmonellosis	*Salmonella sp.*
Bacterial dysentery	*Shigella sp.*
Cholera	*Vibrio cholerae, Vibrio cholerae type eltor*
Stomach and intestine catarrhs	Enteropatogenic *Escherichia coli, Klebsiella pneumoniae, Aeromonas hydrophila, Plesiomonas shigelloides, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Campylobacter (Vibrio) fetus subsp. jejuni, Clostridium perfringens, Bacillus cereus*
Yersiniosis	*Yersinia enterocolitica*
Tularemia	*Pasteurella (Francisella) tularensis*
Leptospirosis	*Leptospira sp.*
Skin infections	*Pseudomonas aeruginisa, Mycobacterium (M. balnei, M. phlei, M. marinum, M. kansasii, M. fortuitum, M. cholonei, M. gorgonae*
Bacteremia conjunctivitis, ear and upper-respiratory system infection	*Psudomonas aeruginosa, Pseudomonas cepacia*
Fever (pyrogens)	Gram-negative water rods *(Pseudomonas, Achromobacter, Xantomonas, Moraxella, Acinetobacter)*
Legionnaires disease	*Legionella pneumophila*

Table 5. Intestinal viruses which may be transmitted by water and diseases caused by them

Viruses	**Number of types**	**Diseases**
Poliovirus	3	Palsies, meningitis, fever
ECHO	34	Meningitis, respiratory system diseases, rash, diarrhea, fever
Coxsackie A	23	Herpangina, respiratory system diseases, meningitis, fever
Coxsackie B	6	Cardiac muscle inflammation, innate heart defects, rash, fever, meningitis, respiratory system diseases, pleurodinia
Enteroviruses	4	Meningitis, encephalitis, respiratory system diseases, acute hemorrhage conjunctivitis, fever
Hepatitis virus, type A	1	Hepatitis type A
Norwalk virus	1	Epidemic diarrhea, fever
Parvovirus	3	Accompany the respiratory system diseases
Adenoviruses	41	Respiratory system disease, eye infections, diarrhea
Rotaviruses	4	Epidemic diarrheas (mainly among children)
Reoviruses	3	Respiratory system diseases

Table 6. Waterborne diseases caused by protozoans

Pathogenic protozoa	Disease	Symptoms
Giardia lamblia (flagellates)	giardiosis	Chronic diarrhea, stomach cramps, flatulence, weight loss, fatigue
Cryptosporidium parvum (sporozoa)	cryptosporidiosis	Stomach aches, loss of appetite, watery diarrhea, weight loss
Entamoeba histolytica (amoebae)	amoebiosis (amoebic dysentery)	Anywhere from slight to acute diarrhea, fever with shivers
Acanthamoeba castellani (amoebae)	amoebic meningoencephalitis	Symptoms from the central nervous system
Naegleria gruberi (amoebae)	amoebic meningoencephalitis	Gets into the brain of swimmers through the nose, causes acute symptoms of meningitis and encephalitis ending in death
*Balantidium coli (*ciliates)	balantidial dysentery	Hemorrhage diarrhea caused by an ulceration of the large intestine

Table 7. Parasitical warms in the human body

Parasite	Symptoms
Human ascarid - *Ascaris lumbricoides (Nematoda)*	Ascariasis. Nematodes' larva causes inflammation reactions in various parts of the body. Sometimes it breaks up the pulmonary alveolus. If the intestines contain a lot of ascarids it may cause intestinal obstruction or a puncture causing damage to the abdomen lining.
Whipworm -*Trichiuris trichura (Nematoda)*	Trichomoniasis disease is caused by nematodes living in human caecum and the large intestine. It creates changes in the mucosa and at high infestation a serious loss of mucous membrane. Sometimes appendicitis may occur.
Spiny – haeded worms (*Acanthocephala*)	Ascanthocephaliasis disease is caused by invertebrates which incorporate only parasitic forms. The parasites live in the intestines of all vertebrate representatives. In water environment their hosts are usually crustacean. The disease manifests itself by inflammation of the digestive system and its physical damage.
Tapeworms - *Taenia saginata, T. solium* (*Cestodes*)	Parasites develop inside the intermediate host until reaching the larva stage called the cysticercus and infect humans who are its final host. Tapeworms live in the small intestine causing nausea, chronic dyspepsia, stomach aches and weight loss.
Flukes - *Schistosoma mansoni* (*Trematodes*)	Schistosomatosis- caused by *Schistosoma mansoni* manifests itself by the ailment of the digestive system, intestine mucosa inflammation and cirrhosis of the liver.

2.6. Water health standards

Why do we control the sanitary quality of water?

The possibility of infection by water imposes a constant need to control the hygienic-sanitary quality of not only drinking water, but also that of swimming pools and surface waters. Water gets infected by pathogenic bacteria excreted by ill people and carriers (people who keep excreting pathogens with faeces long after they have suffered from an illness). Pathogenic microorganisms are present in sewage and surface waters in lower numbers than other microorganisms. Therefore, they are more difficult to detect than the plentiful saprophytic bacteria. Consequently, much more complex diagnostic methods need to be used in order to detect them.

What are indicator microorganisms?

Current norms are based on indirect inference about the presence of pathogenic microorganisms relying on the number of indicator microorganisms, which permanently live in human and animal digestive tracts as saprophytes. Their presence indicates that the water is polluted with faecal matter and, consequently, there is danger of contamination with pathogenic microorganisms.
Bacteria which serve as sanitary indicators should meet the following conditions:
a) they must be constantly present in the human digestive tract so that they allow the detection of the water's contamination with faecal matter
b) the number of indicator bacteria within the intestine and faeces should be high
c) among them, there should be non-spore-forming bacteria as it enables the detection of 'fresh' faecal-matter water pollution
d) their identification must be possible with readily available methods
f) their life span in the external environment should be longer than that of pathogenic bacteria
g) they should not be able to reproduce in a water environment under natural conditions

What types of indicator bacteria are utilized to assess the health quality of water?

In routine laboratory work, which conducts sanitary-epidemiological supervision, it is impossible to constantly monitor water for all pathogenic and potentially pathogenic microorganisms, which may be found in water.

Therefore, routine monitoring concentrates mainly on detecting bacteria that indicate faecal contamination of water (Table 8). The sanitary quality of water may be checked by utilizing the saprophytic microflora that occupy the human large intestine. The following indicators of water contamination have been adopted:

- coliforms
- faecal coliforms
- faecal streptococci
- bacilli of *Clostridium* genus, sulphite-reducing bacteria

and in some instances:
- staphylococci – coagulase positive
- *Pseudomonas aeruginosa*

Coliforms

Bacteria of the *coli* group are mainly made up of strains of *Escherichia coli* as well as the genera: *Enterobacter, Citrobacter and Klebsiella.* They are detected on media containing lactose at 37°C.

Faecal coliforms (thermotolerant) are mainly strains of *Escherichia coli* and only some of the strains of *Enterobacter, Citrobacter* and *Klebsiella,* which have an ability to ferment lactose at 44°C.

The presence of coliforms or faecal coliforms in a water sample indicates relatively recent contamination of water with faecal matter, sewage, soil or with decaying plants. For most types of waters a quantitative determination of both groups of coliforms is recommended.

Faecal streptococci

While in a water environment, faecal streptococci are characterized by a slightly longer period of survival and resistance to most disinfecting products than the coliforms. Faecal streptococci include microorganisms of *Enterococcus and Streptococcus* genera, which belong to the serological group of Lancefield D. Detection of faecal streptococci in a test sample, significantly exceeding the *coli* group bacteria, may suggest water contamination with animal faecal matter or sewage from animal farms.

Bacilli of *Clostridium* genus

The detection of sulphite reducing bacteria (mainly strains of C*lostridium perfringens*) may suggest less recent contamination with faecal matter; their endospores are able to survive for many years in unfavourable conditions. Sulphite reducing clostridia are a good indicator of properly conducted water treatment processes - coagulation, sedimentation, and filtration.

Endospores of these bacteria as well as the cysts of parasitic protozoa (*Cryptosporidium parvum, Giardia lamblia*) ought to be eliminated in those stages of water treatment, because they are especially resistant to the disinfecting agents.

Conducting analysis of a water sample, in order to detect bacteria of the *Clostridium* genus, is technically less complicated than searching for parasitic protozoa and it ensures that the treated water is free from protozoa and from the eggs of pathogenic worms (Helminthes).

Pseudomonas aeruginosa

Currently, detection of *Pseudomonas aeruginosa* bacteria in drinking water, running water, swimming pools and surface waters is recommended in addition to the above elements of sanitary analysis.

They are Gram-negative rods that do not produce spores. Their characteristic trait is the ability to produce a blue-green pigment - pyocyanin as well as a fluorescent pigment – fluorescein.

Representatives of this species were isolated from human faeces and in cases of infection - from urinary tracts, inner ear, suppurating wounds etc. These bacteria pose a potential pathogenic danger for both humans and animals. In addition, they are widely distributed in surface waters and soil. It is also important that the species may live in chlorinated water because it is, to some extent, resistant to disinfection.

Staphylococci

The *Staphylococcus* genus is mainly used to assess sanitary quality of swimming pools. Recreation waters are the cause of infections of respiratory tracts, skin and eyes. For this reason microbiological analysis based on standard indicators (coliforms) is insufficient. Some researchers have recommended *Staphylococcus aureus* to be used as an additional indicator of sanitary quality of recreational waters, because its presence is associated with human activity in these waters.

Total number of bacteria

In routine analysis the total number of bacteria present in 1 ml of water is also determined by an agar plate method. One set of plates is incubated at 37°C for 48 h (mesophilic bacteria). Another set of plates is incubated at 22°C for 72 h (psychrophilic bacteria). After incubation the colonies are counted and the amount of cfu/ml (colony forming units) can be calculated.

The total number of psychrophilic bacteria
Non pathogenic water bacteria grow mainly at lower temperatures. It is important that Gram-negative bacteria in water produce lipopolysaccharides in their cell wall which can be toxic – like endotoxins of pathogenic bacteria. Because of this, their numbers in water should be constantly monitored.

A large increase in their numbers is evidence of the presence of easily available organic compounds in the water. Theoretically, the presence of 0.1 mg organic carbon in water can result in an increase of bacteria up to 10^8 cfu in 1 ml.
Phosphorus is also a factor which stimulates the growth of microorganisms. Adding even small amounts of this element (<50mg/l) causes 10 times the acceleration of bacterial growth in a water treatment plant.

The total number of mesophilic bacteria
More dangerous are high numbers of bacteria growing at 37 ^{0}C, because among this high population, pathogenic forms may be found which are dangerous for human health.
High number of bacteria in samples of water can prove that water treatment processes proceed badly or that polluted water is siphoned.

What is the cause of increasing levels of total number of bacteria in water?

An increase in the total number of bacteria in water samples can also be proof of development of microorganisms on inner surfaces, especially on pipe-joints, seals and of the creation of the layer called a biofilm. Biofilms of microorganisms are of concern because of the potential protection of pathogens from the action of residual disinfectant in the water and the regrowth of indicator bacteria such as coliforms.
High number of total bacteria is an indicator of potential pathogens and one should start looking for the source of pollution and taking proper actions. Sometimes additional chlorination is needed eg. for drinking water over 0,2 mg Cl_2/l. In some cases changes to the construction of the water supply system and removal of the biofilm are effective protection against the excessive level of microorganisms in water.

Drinking Water Criteria in Poland

Assessment of bacteriological quality of drinking water in Poland is based on the level of the groups of indicators such as: *coliforms, faecal coliforms, enterococci* and *Clostridium perfringens.* In 100 ml of water present in a water-pipe network not a single bacterial cell considered as an indicator can be present (Table 8). Similar drinking water criteria are required in the European Union (Table 9).

The second important criterion is a total number of psychrophilic and mesophilic bacteria in 1 ml of water. The level of psychrophilic bacteria should not be more than 100 cells in 1 ml of water, whereas mesophilic bacteria must be lower than 20 cells in 1 ml of water.

Bacteriological quality of recreation waters (swimming pools) is judged on the basis of the level of indicators such as: *coliforms, fecal coliforms, staphylococci* (coagulase positive) and the total number of mesophilic bacteria. In 100 ml of water present in swimming pools no fecal coliforms and maximally 2 cells of coliforms and staphylococci can be present. The level of mesophilic bacteria should not be more than 100 cells in 1 ml of water (table 8).

Table 8. Water quality criteria in Poland

Indicator	Highest acceptable value of indicator			
	Supplied water		Water from swimming pool	
	Number of bacteria	Volume of sample [ml]	Number of bacteria	Volume of sample [ml]
Escherichia coli or *faecal coliforms* (termotolerant)	0	100	0	100
Total coliforms	0	100	2	100
Enterococci (faecal streptococci)	0	100	x	x
Sulfite reducing clostridia (*Clostridium perfringens*)	0	100	x	x
Staphylococci coagulase positive	x	x	2	100
Total number of mesophilic bacteria (37 °C)	20	1	100	1
Total number of psychrophilic bacteria (22 °C)	100	1	x	x

Table 9. Comparison of drinking water criteria in Poland and European Union [Council Directive 98/83/EC]

Indicator	European Union	Poland
Escherichia coli	0/100 ml	0/100 ml
Fecal streptococci	0/100 ml	0/100 ml
Sulfite-reducing clostridia	0/100 ml	0/100 ml
Total number of mesophilic bacteria (37 °C/24 h)	20/1ml	20/1ml
Total number of psychrophilic bacteria (22 °/72h)	100/1ml	100/1ml

2.7. Wastewater treatment

What is wastewater?

Wastewater (sewage) is polluted water which includes all harmful liquid, solid or gaseous substances introduced into waters or soil that may lead to a contamination of surface or underground waters. Sewage also includes: used-up liquids, solutions, colloids, suspensions, radiocontaminated waters, saline waters, heated cooling waters, precipitation waters or waters which contain various impurities from urban and rural areas.

Types of sewage

A. classification based on origin
- domestic sewage contains large amounts of faecal matter, plant and animal wastes, surface-active agents, urea. The sewage comes from households, public lavatories and industrial facilities posing a serious hygienic and epidemiological threat,
- industrial (technological), evolve during all types of industrial processes (manufacturing and processing),
- precipitation (rain and melt waters) contain various atmospheric impurities (dusts, microorganisms, gaseous substances), surface run-offs, streets and paved surfaces run-offs (oils, liquid fuels, bacteria, small particle suspensions), microbiological impurities (bacteria, viruses, fungi),

B. classification based on harmfulness
- directly harmful
- indirectly harmful (lead to a decrease of oxygen in water below the essential organisms' requirement)

C. classification based on contamination stability
- degradable - organic substances that undergo chemical transformations to form simple compounds,
- non-degradable - substances that do not yield to any chemical transformations and are not decomposable by microorganisms,
- stable - substances which only slightly undergo biological decomposition and stay in the habitat in a unchanged form for a long time.

D. man made
- urban and domestic - source: food serving facilities, hospitals, houses and apartments posing a hygienic and epidemiological threat,
- rural - source: farms, pig fattening houses, animal farms, intensively fertilized fields,
- industrial - source: manufacture and processing of all branches of industry; this type of sewage is a major source of toxins,
- radioactive - source: scientific and health facilities, nuclear reactors; such types of wastes are especially dangerous to the habitat therefore they require special storage methods.

What pollutants do municipal sewage contain?

Sewage is characterized by the following groups of organic and non-organic impurities:
- soluble substances,
- settling suspensions,
- liquid suspended suspensions.

Chemical impurities contained in sewage may be divided into:
- dissolved mineral substances (sulphates, chlorides, acid and neutral carbonates, calcium, magnesium, sodium, bases, nitrates, phosphates etc.),

- soluble gases (oxygen, hydrogen sulphide, carbon dioxide, nitrogen),
- soluble organic substances (proteins – about 40-60%, carbohydrates – about 25-50%, oils and fats – about 10%)

One further classification of pollutants in sewage is as follows:

a) physical impurities
b) chemical impurities
c) biological impurities

a)
physical pollutants of sewage are characterized by properties which can be detected by the senses (sight, smell). The properties of physical pollutants are: suspension, cloudiness, colour, smell, temperature.

b)
organic pollutants are defined by three common parameters:
BOD (biochemical oxygen demand), COD (chemical oxygen demand), TOC (total organic carbon).

BOD – determines the amount of oxygen required by bacteria in order to biologically oxidize decomposable organic compounds in aerobic conditions in a temperature of 20 ^{0}C. About 50% of pollutants are oxidized by microorganisms over a period of three days. Five days as the representative period is assumed to determine the characteristic of biochemical oxygen demand (BZT_5).

COD – specifies the amount of oxygen required to oxidize organic compounds chemically.

TOC – specifies the amount of carbon contained in organic compounds.

c)
biological pollutants include microorganisms (viruses, bacteria, fungi), eggs of helminthes.

What are biogenic pollutants and what dangers are connected with them?

Biogenic pollutants are made up of mineral salts of elements which are essential for the development of living organisms. The basic ones are the compounds of phosphorus and nitrogen. After introduction into lakes and rivers, the above compounds increase their fertility causing eutrophication.

Eutrophication is a term that describes a complex of unfavourable symptoms connected with over-fertilization. Urban sewage contains phosphates from human excrements, washing detergents and liquids, food waste, food additives and other products. Another significant source of phosphate pollution of water is sewage from the agricultural industry. The presence of phosphorus in sewage introduced into water along with nitrates and nitric dioxides causes increased development of algae in both lotic and lentic waters. Increased eutrophication has been considered to be hazardous to water reservoirs as a consequence of uncontrollable growth of plant biomass.

What are refraction pollutants?

Refraction pollutants are those which do not, or only to a minimal extent, undergo biological decomposition by microorganisms. Some of them demonstrate characteristics of dangerous poisons e.g. heavy metals, PAH (polycyclic aromatic hydrocarbons), PCB (polychlorinated biphenyls), dioxins, pesticides, nitrosamines.

Why is sewage subjected to purification?

Elimination of contamination from industrial and municipal wastes, prior to their reintroduction to a receiving body of water, results from a need for rational management of water supply, environment protection and adequate sanitary conditions. Introduction of pollutants, depending on the watercourse, may decrease the water's physical, chemical and sanitary conditions or even cause the disturbance of biological balance.

What are the main objectives of the wastewater treatment process?

The objectives of the wastewater treatment process are:

- lowering the content of organic carbon including compounds which are difficult to biodegrade as well as the toxic, mutagenic and carcinogenic ones,
- reduction of biogenic substances: mineral salts of nitrogen and phosphorus,
- elimination or inactivation of pathogenic microorganisms and parasites.

What methods of the wastewater treatment are there?

Depending on the type of pollutants, there are different methods of purification used prior to reintroduction into a receiving body of water. The methods are classified as follows:

- mechanical – in this method only non-soluble pollutants are removed by utilizing the following processes: gravitational and centrifugal sedimentation, flotation, source filtration, separation in hydrocyclones, which allow the removal of organic and mineral suspensions as well as floating bodies;
- physical-chemical – utilizes the following operations and processes: coagulation, co-precipitation, sorption, ion exchange, electrolysis, reverse osmosis, ultrafiltration;
- chemical – utilizes neutralization, oxidation, reduction;
- biological – consists of sewage purification (elimination of organic pollutants as well as biogenic and some refraction compounds) during biochemical processes of mineralization conducted naturally by microorganisms in a water habitat (e.g. sprinkling of wastewater onto agricultural lands), or in special devices (on trickling filters or activated sludge).

What are the typical stages of sewage treatment?

A typical process of sewage treatment consists of four stages of purification:

- mechanical (stage I of purification),
- biological (stage II of purification),
- elimination of biogenic compounds (stage III of purification),
- water renovation (stage IV of purification).

Stage I of purification, primary treatment the so-called initial or mechanical purification. The goal of this stage is the removal of solid impurities. This stage is considered to be the preparation of sewage for further purification. By utilizing simple mechanical operations the following impurities are removed during the first stage:

- floating solid impurities,
- settling suspensions,
- oils and fats.

Stage II of purification, secondary treatment includes biological purification, which leads to the biodegradation of soluble organic impurities, colloidal systems and suspensions not removed during the first stage. The intensification of purification processes is obtained by utilizing trickling filters and activated sludge.

Stage III of purification, tertiary treatment includes processes used to thoroughly clean sewage. The largest impurities removed during this process are the biogenic compounds (compounds of phosphorus and nitrogen).

The nitrogenous compounds are removed during the process of biological nitrification and de-nitrification, whereas the compounds of phosphorus are eliminated by a process of chemical precipitation. The role of thorough cleaning of sewage in this stage is the prevention of water eutrophication.

Stage IV of purification (water renovation) includes the processes of residual sewage removal, which are left over from the previous stages of purification. Water regeneration involves a set of methods which confer the properties of natural water onto the sewage so that it can be utilized in industrial facilities. Water regeneration allows the recycling of sewage, which is a significant element in water resource management, especially in regions low in water. There are several systems of water regeneration, from very simple ones, that use rapid filters or straining through microsieves, to very complex physical-chemical processes: coagulation, membrane processes and disinfection, sedimentation, expelling of ammonium, recarbonization, absorption, ion exchange, and water demineralization.

2.7.1. Biological methods of wastewater treatment

In biological methods of sewage treatment, bacteria which form zoogleal clusters in sewage play a crucial role. Methods of biological purification of sewage consist of inducing the enzymatic processes of saprophytic microorganisms that include partial oxidation of organic substances (sources of carbon) contained in sewage as well as their partial assimilation by microorganisms. As a result of these processes, an increase in cell mass of the active microorganisms occurs. Microorganisms flourish when the ratio of three basic elements C:N:P = 100:10:1.

The biological processes of purification can be divided into natural and artificial, depending on where the processes take place - whether they occur in natural conditions or are intentionally triggered in specially designed artificial equipment.

Biological purification can be conducted in oxygen rich, oxygen-poor or oxygen-free conditions. It is a process of oxidation and mineralization of organic compounds from sewage using micro- and macroorganisms.

During the process of biological purification the following phenomena take place:

- breakdown of organic substances down to CO_2, H_2O, NH_3 (dependent on pH)
- nitrification (oxidation of NH_3 by *Nitrosomonas* bacteria down to nitrites, and then by *Nitrobacte*r bacteria down to nitrates),
- denitrification (transformation of nitrates to gaseous nitrogen N_2)

A. Natural methods

Natural methods of wastewater treatment include: purification in soil, field and forest irrigation (the method of irrigation and filtration fields) and soil filters.

Purification in soil

Biological purification in a field soil consists in irrigation of a field with sewage. Biogenic substances contained in sewage lead to an average of 20% yield increase. A field used for agricultural purposes can receive an annual dose of 600 mm effluent per annum. After spreading, the sewage seeps into the soil and the contents of impurities are absorbed by the soil particles.

Prior to introducing sewage onto the fields, sewage undergoes mechanical purification (screens, sand traps, primary settling tank) and is disinfected. Moreover the irrigated soil is checked for the content of metals. Field irrigation can be conducted only during the period of plant vegetation and the amount of the applied sewage has to be altered at different times. In the winter sewage is purified on filtration fields.

After some time, the absorbed organic compounds and microorganisms create a microscopic film around the particles of soil and the surface soil layer works like a biological filter. The final products of the mineralization process taking place in this layer, act as fertilizer for the soil.

Only a limited amount of sewage can be purified by this method, otherwise the field becomes excessively loaded with sewage. In such situations, oxygen-free processes are triggered, that are accompanied by the formation of toxic substances and odour release causing the plant growth to stop.
Due to sanitary reasons, prior to irrigation, sewage must be cleared of any helminth eggs. During the infiltration in soil, sewage gets purified and then carried over to a receiving body of water by a drainage system.

Soil filters

Purification by soil filters consists of spreading waste upon the surface of soil that leads to its biological purification. Most often non-cultivable fields are utilized for such forms of purification. The lack of agricultural use allows utilization of greater amounts of sewage (annual dosage of sewage may go as high as 3000 mm/a). Loose and sandy soils with a grain diameter of 0,2-0,5 mm, with strata thickness between 1.5-2.0 m and with a low level of underground water are best suited for this purpose. The field is divided into drying beds (about 0,5 hectare area).
Not cultivating the soil can result in greater amounts of sewage being purified.
Prior to pouring out the sewage, it has to be mechanically cleaned, in order to eliminate the oily suspension that clogs up the source. The drying beds are flooded with wastes (thickness 5-10 cm) every 0,5-4 days.
Purified waste is drained by a drainage system installed in the ground. After some time, the soil filters lose the ability to purify and have to be periodically excluded from operation in order to regenerate.

Sewage may undergo purification in the winter when it is upon the filtrating fields. The number of active plots is then lowered in order to minimize losses, and instead the depth of the flooding increases to 20-30cm. The surface of the sewage covers with ice, under which the sewage is supplied and undergoes the process of purification while decreasing the quality of the outlet.

Sewage ponds

Sewage ponds are earth reservoirs, in which the process of biological purification occurs naturally (utilizing microbes), thus they are used in smaller towns, where the number of inhabitants does not exceed 20,000.

Sewage ponds are either natural or artificial ground reservoirs, in which solar radiation reaches the bottom. Prior to introduction of sewage into the pond sewage has to be preliminarily cleared of suspended matter. Sewage ponds usually consist of a series of ponds: bacterial, algal and crustaceal.

Oxidation of organic compounds by bacteria takes place in the bacterial pond, which leads to their mineralization, i.e. transformation into non-organic compounds known as biogenic salts.

Sewage purified in that way is then directed to algal ponds, where algae flourish on it, assimilating the mineral compounds that evolved during the process of biodegradation.

The final stage of such purification is conducted in the crustacean pond, where algae-eating crustaceans flourish. Such systems of purification allow the elimination not only of organic substances but also the excess biogenes, whose presence in the receiving body of water could cause eutrophication and consequently water blooming which lowers the oxygen content in water.

This pond can be used for fish and duck breeding without a need for artificial feeding.

Hydrobotanic purification and the idea behind it

Hydrobotanic purification consists of utilization of self-purifying processes that take place in waterlogged ecosystems, thus it belongs to so-called wetland systems.

Purification is a result of the co-operation of soil microorganisms and boggy plants. Microorganisms decompose the organic compounds contained in sewage, turning them into non-organic compounds, whereas plants absorb the produced mineral compounds creating a plant biomass. The adsorption of impurities by the particles of soil is improved, due to very small mineral particles (silt) present in the substrate.

This type of treatment unit utilizes wetland vegetation (common-reed, reed-mace, basket willow, etc) that has a high requirement for food, thus it absorbs large quantities of mineral salts. As a result, the vegetation desalts the sewage and does not lead to eutrophication of water reservoirs.

What types of hydrobotanic purification plants are there?

There are three types of hydrobotanic purification plants:

- **soil-plant filters -** are the types of filters with horizontal (most common and the longest in use), vertical or combined flow; mainly sandy with rooted boggy vegetation (common-reed, reed-mace, schrubby willows).
- **shallow reservoirs with rooted vegetation -** these include water reservoirs or ducts of depths between 10-50 cm, they are occupied by boggy vegetation (common-reed, reed-mace, sedge).
- **sealed reservoirs with floating vegetation -** ponds of depth between 1-2 m, with sealed bottoms and side walls, with floating vegetation – in our climatic conditions it is duckweed – *Lemna minor*. Exploitation problems are connected with an even spread of duckweed throughout the surface of the pond and removal of the rapidly growing plants. In the winter, due to lack of vegetation, the purification plant plays the role of a normal pond.

B. Artificial methods of wastewater treatment

Trickling filters

The treatment of sewage by trickling filters is conducted in reservoirs filled with loose, grainy and porous material. Sewage is sprayed upon the upper layer of the bed with sprinklers and then left to seep through its content (Fig. 2.23-24).

A mucous biological film forms upon the content of the bed. The film is composed of microorganisms such as: bacteria, protozoa and fungi. The role of the filter involves a constant supply of sewage and its flow through the trickling filter while maintaining contact with the biological film. During the flow, the sewage undergoes mineralization as a result of aerobic decomposition by microorganisms.

The biological film is initially composed of zoogleal bacteria which produce mucous sheets. With time, the composition of species of the mucous membrane changes due to their succession. Besides bacteria, the following appear: fungi, protozoa, annelida and fly larvae. Depending on the amount of treated sewage, the trickling filters may be subdivided into percolating and flushing filters.

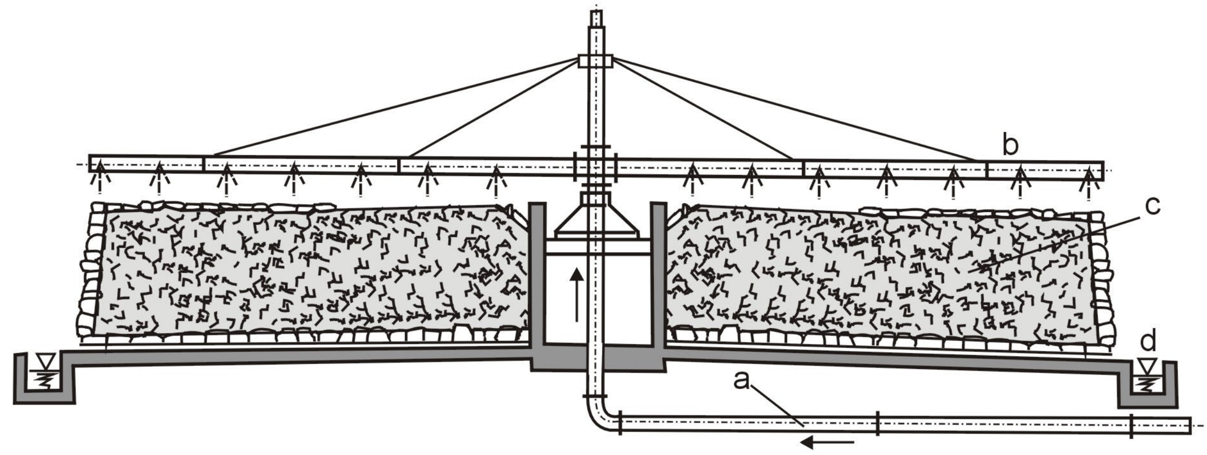

Fig. 2.23. Trickling filter:
a) inflow, b) percolating system, c) filter packing, d) effluent

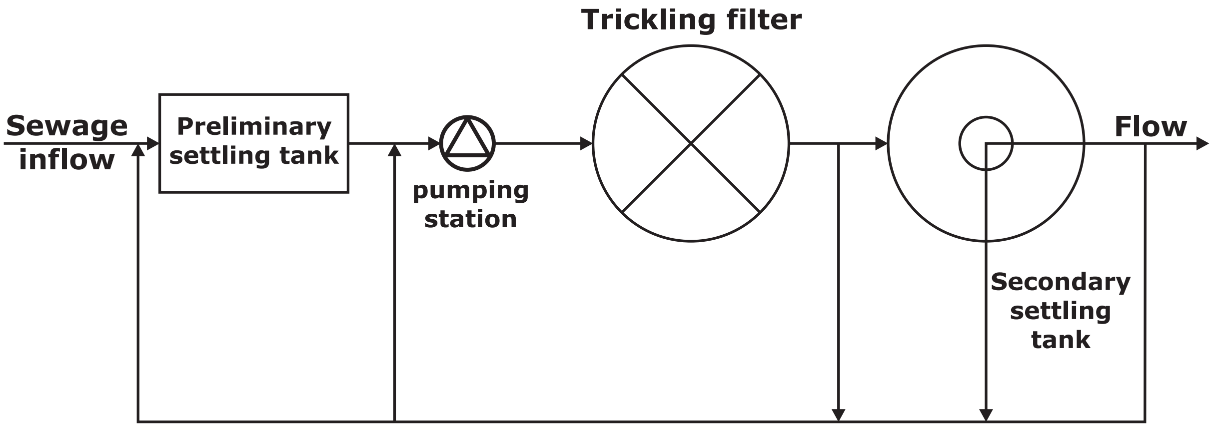

Fig. 2.24. The trickling filter process

Depending on the amount of the organic load the following types of biofilters are distinguished:

- Low loaded - may be filled with natural or artificial material. The supplied organic material is less than 0.4kg $BZT_5/m^3 \cdot d$. In percolating filters the film is more developed and the biological process of decomposition is almost complete. In the final phase of purification, intensive processes of nitrification occur, which lead to an increase of nitrates in a run-off to the secondary settling tank.
- Mid-loaded - are filled with natural-synthetic material and work with a load between 0.4-0.65 kg $BZT_5/m^3 \cdot d$. In order to ensure an adequate concentration of the supplied sewage the recirculation of part of the purified sewage is utilized with this type of filters. The reduction of organic compounds upon these filters is adequate, and the processes of nitrification partially occur. The introduction of additional processes of purification is not necessary.
- High loaded (flushed) - are filled with natural-synthetic material, the filter is loaded with: 0.65-1.6kg $BZT_5/m^3 \cdot d$. In flushing filters, the intensity of sewage flow is greater, however the biofilm is composed almost entirely of bacteria and does not develop as much as in the above stated case. Flowing sewage washes out used and dead biological material from the filter. The washed out material is transported in the form of flocy sediment. Only a partial mineralization of organic compounds occures on that type of filter and the nitrification process is inhibited. A low content of nitrates in effluent from filters testifies to partial mineralization of organic compounds. In complex systems, after these types of filters, re-purification is utilized, as the quality of the purified sewage does not usually meet the required standards.

Activated sludge

The process of activated sludge relies on sewage purification by freely suspended matter. It consists of producing 50-100 μm flocs with highly developed surface areas. The floc is made up of brown or beige mineral nucleus, while on its surface it contains heterotrophic bacteria within the mucous envelopes. The method of activated sludge requires delivery of oxygen into the substrate for bio-oxidation of organic pollutants, which should be >0,5 mg/dm^3 in order to ensure proper oxygen conditions for the bacteria.

Activated sludge characteristics

Activated sludge is a type of flocculent suspended matter created during the aeration of sewage.

Treating sewage with activated sludge consists of mineralization of organic compounds, conducted mainly by bacteria and following the same biochemical processes as observed in self-purification. However, the speed of the process is much greater. This results from the fact that the conditions of intensive aeration, triggered during sewage flow through aeration tanks are conducive to the development of impurity-decomposing bacteria. Agglomeraters (flocs) which consist of heterotrophic bacteria coagulated with mucous, form during the process of aeration in aeration tanks (flocculation). The floccules absorb impurities contained in sewage, whereas microorganisms in floc decompose the absorbed substances.

Activated sludge has a spongy, loose structure, made of small openings of various shapes. Undisturbed floccules easily settle and thus allow the separation of the activated sludge from sewage.

Biocenosis of activated sludge is, for the most part, composed of heterotrophic bacteria. In small percentages - and only under particular conditions and in some arrangements - it's made up of chemolithotrophic bacteria, especially nitrifying bacteria. The most common species of activated sludge are: *Zooglea ramigera* (fig.25), *Pseudomonas fluorescens, Pseudomonas putida* as well as bacteria of *Achromobacter, Bacillus, Flavobacterium and Alcaligenes* genera. The process of selection occurs naturally. The conditions in an aeration tank, especially the chemical composition, pH-value and air conditions, are the determining factors for the diversity of the bacterial complex.

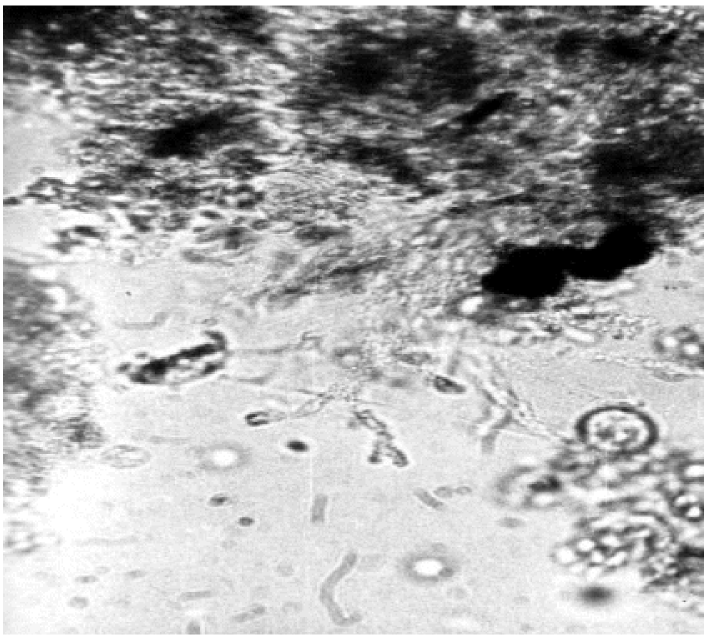

Fig. 2.25. *Zooglea ramigera* in activated slugde

In unfavourable conditions (overloading of aeration tanks with easily available substrates, high oxygen deficit) excess development of flocs occurs causing the so-called active-sludge swelling. There are two distinquishable types of swelling: fibrous and non-fibrous swelling.

Fibrous swelling is caused by excess filiform bacteria (*Sphaerotilus natans, Beggiatoa alba* or *Thiothrix nivea*) or fungi development. Non-fibrous swelling is caused by bacterial development, which produce excess amounts of mucous.

Active sludge biocenosis is made up of not only bacteria but also protozoa, nematodes and rotifers (Fig. 2.26-29). Even though these microorganisms do not play a major role, their presence is equally important.

Fig. 2.26. Ciliata - *Epistylis umbellaria* in activated sludge

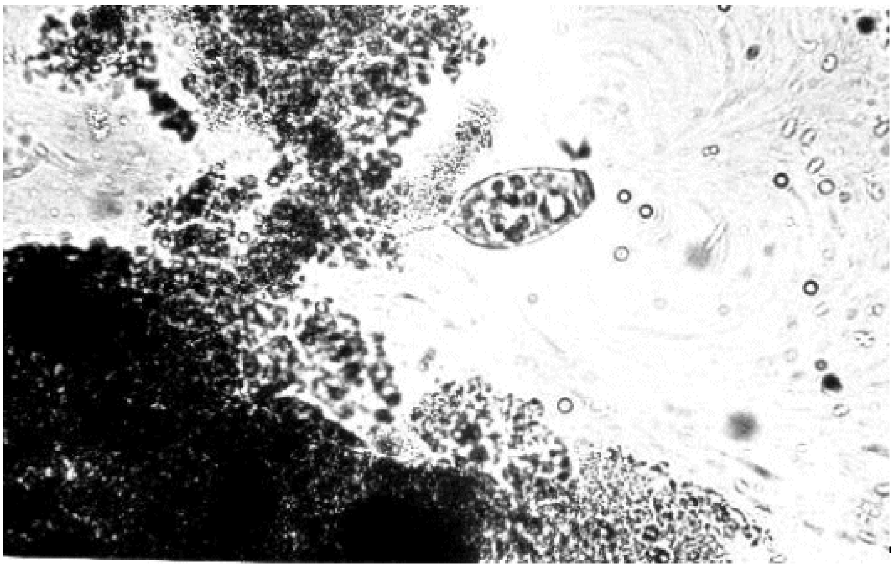

Fig. 2.27. Ciliata – *Opercularia* sp. in activated sludge

Fig. 2.28. Rotatoria - *Rotifer vulgaris* in activated sludge

Fig. 2.29. Nematodes – *Plectus* sp. in activated sludge

Protozoa feed upon bacterial cells forcing them to reproduce quickly, which essentially make them an important renewal and reactivating factor of the activated sludge. The most common protozoa are: *Vorticella, Carchesium* and *Opercularia* as well as *Anthophysa, Oxytricha, Stylonychia* and *Lionotus.*

There is an inverse relationship between flagellates and ciliates within activated sludge. While a large number of flagellates indicate an overload of sludge, the presence of ciliates goes to show it is functioning properly. During the course of sewage purification with activated sludge a characteristic succession of biocenosis is observed.

Activated sludge process

Sewage is directed to aeration tanks filled with activated sludge (thick suspension of microorganisms) after its mechanical purification (Fig. 2.30). The content of the aeration tank is constantly aerated in order to provide an adequate amount of oxygen, to keep the activated sludge in a suspended state and to ensure its constant mixing.

The aeration tank is a device, in which the development of the activated sludge results from continuous cultivation. There is a state of equilibrium between the rate of sewage inflow, concentration of nutrients, bacterial reproductive rate, and the rate of the sewage outflow containing some activated sludge in it.

During the time of contact of sewage with the activated sludge, the decomposition processes occurring simultaneously enable the development of activated sludge biomass.

Separation of purified sewage is done in a secondary settlement tank. Both sedimentation and clarification of the purified sewage, which is then carried off to a receiving body of water, occurs in the device.

Activated sludge may be used again for purification; it is then recycled into the aeration chamber. However, quite often, before reuse, the sludge is directed to a regenerative chamber, where it is aerated in order to bring back its particular physiological properties.
When the sludge collected in a secondary settling tank is not recycled, then, as an excess sludge, it is removed and subjected to additional processing.

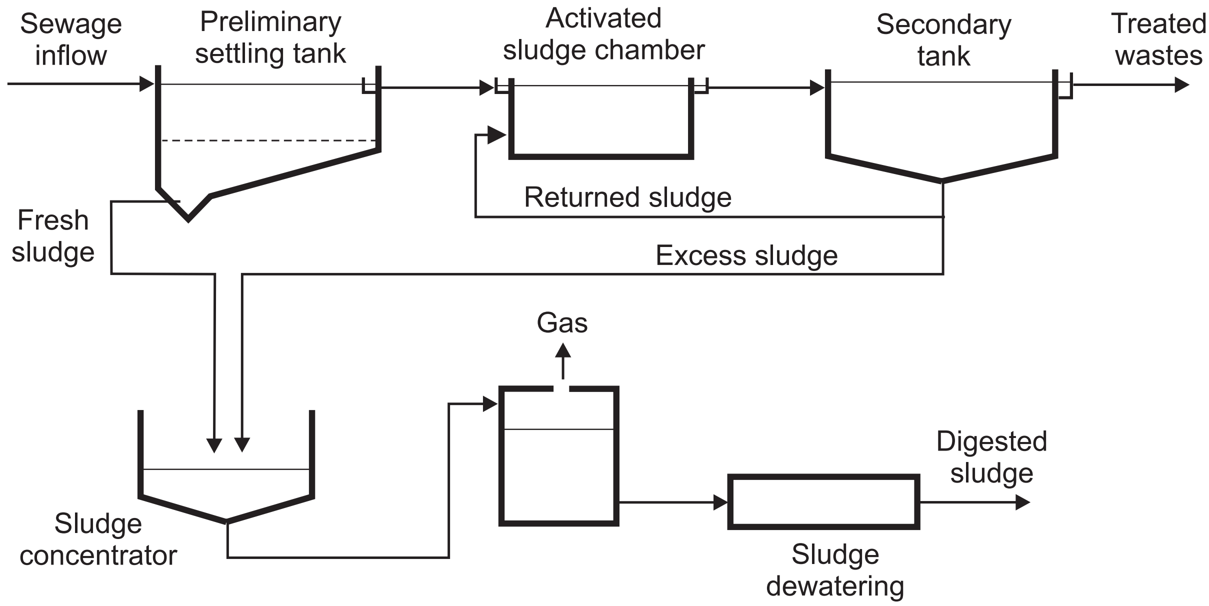

Fig. 2.30. The activated sludge process

2.7.2. Methods of chemical wastewater treatment
Purification of industrial sewage that contains mineral and organic compounds, and heavy metals, utilizes physical-chemical and chemical methods. They include the following processes: neutralization, coagulation, oxidation, reduction, sorption, flotation, membrane processes, extraction, electrolysis, distillation.

Neutralization. It is a process of chemical neutralization of sewage in relation to the pH. Depending on the make-up of sewage and the type of the reacting substance used, neutralization may be accompanied by a chemical process of precipitation and co-precipitation.

Neutralization may be conducted by mixing acidic sewage with bases. Hydroxides are substances most often used in the process of neutralization: NaOH in the form of 20-30% solution, $Ca(OH)_2$ in the form of 5-15% milk of lime, Na_2CO_3 in solution form $CaCO_3$, $MgCO_3$, MgO, dolomite in the form of a grainy filter. Mineral acids are used for the neutralization of basic sewage: H_2SO_4, HCl, H_3PO_4 in the form of solutions as well as CO_2 in the form of a clear gas.

Coagulation is a process of binding colloidal particles and the suspension into clusters of particles called the agglomerates, which results in precipitation of the sediment in the form of coagulate. The factors which most often cause coagulation are: addition of an electrolyte solution to lower the electrolytic potential, addition of colloids of an opposite charge into the colloidal particles, creation of metal hydroxides that absorb ions, colloids and suspensions.

Oxidation. An oxidation process is conducted in order to remove organic compounds, non-organic compounds and microorganisms from sewage. The reacting substances used in oxidation are: chlorine, chlorine oxidizing compounds ($NaOCl$, $Ca(OCl)_2$, chlorinated lime, chlorine dioxide, ozone.

Reduction. The process of reduction used in sewage purification mainly concerns chromium. Chromium salts (VI) are toxic, carcinogenic, bacteriocidal and are irritants to skin. Its bacteriocidal properties slow down the process of water self-purification. Reduction of chrome from oxidation state of 6+ down to 3+ is conducted through reduction and precipitation of hydroxide, which belongs to a group of barely soluble compounds. Reduction is conducted either chemically or electrochemically.

Sorption. Sorption consists of binding liquid soluble substances to the surface of solids. Depending on the characteristics of the process it may be irreversible (chemiosorption), or reversible - adsorption. The characteristic of the process of sorption is determined by one of the components of force:

- physical sorption - the result of van der Waals forces,
- chemical sorption - the result of valence forces,
- ion sorption - between groups of cations and anions in the structure of the substrate,
- sieve sorption - at the molecular level according to the mechanism of a molecular sieve

Flotation. A process of structural separation consisting of raising the hydrophobic impurities into the foam along with the rising gas bubbles. As a result, the foam formed has a much higher concentration of pollutants than the rest of the sewage.

Membrane processes. These processes consist of separation of particles by flowing through a porous layer (membrane). The following are the types of membrane processes: reversed osmosis, nanofiltration, ultrafiltration, electrodialysis.

Extraction. This consists of transfer of components from one phase of the solution into the second liquid phase (dissolvent). Consequently a solution of the component in the dissolvent is obtained. The required condition for the process is the presence of two liquid phases.

Electrolysis. The process in which electrical energy invokes chemical changes of the electrolyte. As a result of the electrical field the movement of ions toward the electrodes (upon which the process occurs) occurs:

- cathode $Me^+ + e^- \rightarrow Me$ (reduction)
- anode $X^- \rightarrow X + e^-$ (oxidation)

Distillation. Process that utilizes the difference between the composition of a liquid and vapour in the state of equilibrium.

Evaluation

What type of abiotic factors limit the development of microorganisms in water?
What is the trophicity of surface waters?
What is the process called eutrophication?
Can you explain the mutual interactions that exist between individual members of the biological group that inhabit the surface water?
Can you specify what kind of microorganisms live in waters?
What is the role of bacteria in a water environment?
Describe the water self–purification process.
List the types of pathogenic organisms transmitted by water.
What can you say about the Polish health criteria for water?
Explain the difference between natural and artificial biological methods of wastewater treatment.
What do you know about the role of bacteria in activated sludge?

Summary

The biotopes of water microorganisms are the underground and surface waters as well as the bottom sediment. The development of microorganisms in water is influenced by a number of chemical, physical and biological factors. Microorganisms occupy bodies of surface waters in all zones (plankton, periphiton, benthos). A water habitat contains autochthonous and allochthonous microorganisms. Waters become polluted as a result of domestic and industrial sewage disposal into the surface waters. Self-purification of surface waters encompasses complex co-operation of physical and biochemical factors. Some pathogenic organisms can be transmitted by water, therefore it is necessary to control the sanitary quality of waters. The health hazard connected with water pollution is limited by the development of wastewater treatment processes.

Glossary

Aerobe: An organism that requires free oxygen for growth
Anaerobe: An organism that lives and reproduces in the absence of dissolved oxygen, instead deriving oxygen from the breakdown of complex substances.
Activated sludge: Sludge particles produced in raw or settled wastewater (primary effluent) by the growth of organisms (including zoogleal bacteria) in aeration tanks in the presence of dissolved oxygen. The term “activated” comes from the fact that the particles are teeming with bacteria, fungi and protozoa. Activated sludge is different from primary sludge in that the sludge particles contain many living organisms which can feed on the incoming wastewater
BOD: Biochemical Oxygen Demand – the amount of oxygen consumed by water microorganisms in breaking down the organic matter.
Biodegradable matter: Organic matter that can be broken down by bacteria or other microorganisms to more stable forms which will not create a nuisance or give off foul odors.
Micronutrient: An element required by plants and bacteria, in relatively small amounts, for survival and growth. Micronutrients include: Iron (Fe), Manganese (Mn), Zinc (Zn), Boron (B), and Molybdenum (Mo).
Pathogens: Bacteria, viruses, fungi or protozoans which cause disease.
Wastewater: The used water from households and industrial plants. Storm runoff and infiltration water may also be included in the wastewater.
Zoogleal film: A complex population of organisms that form a "slime growth" on a trickling-filter media and break down the organic matter in wastewater

3. Microbiology of air

Contents

Aims

After studying this text you should have an understanding of the specificity of prevalent environmental conditions acting in the air and their influence on survival of microorganisms. It is important to recognise fully the threat associated with biological aerosols. Also you should become familiar with a basic research methods used in studies of microbiological pollutants in air and the sanitary requirements for air quality.

Orientation

In this text, the air is characterized as a transient environment for microorganisms. The transmission of microbes is discussed including the influence of different factors on their survival. Attention is given to threats connected with the aspiration of the microbiologically polluted air and to mechanisms to protect the respiratory system against bioaerosol penetration. How to investigate and evaluate the quality of the air using microbiological methods is also explained.

Prior knowledge

For a full understanding of the contents of this chapter, basic knowledge of biology at a college level, and the fundamentals of general microbiology are required.

Study advice

The student should remember basic knowledge about bacteria, fungi and viruses (their morphology, means of reproduction and environmental requirements).

3.1. The air as an environment of microorganisms

- Air is an unfavourable environment for microorganisms, in which they cannot grow or divide. It is merely a place which they temporarily occupy and use for movement.
- Therefore, there are no metabolic connections occurring between different microorganisms in air (such as in soil or water). As a result they form only a random collection of microorganisms, not a microbiocenosis.
- Microorganisms get into air as a consequence of wind movement, which sweeps them away from various habitats and surroundings (soil, water, waste, plant surfaces, animals, and other), or are introduced during the processes of sneezing, coughing, or sewage aeration.

Why are the air conditions unfavourable for the microorganisms?

There are 3 elementary limiting factors in the air:
- a lack of adequate nutrients,
- frequent deficit of water, threat of desiccation,
- solar radiation.

It is obvious that the first factor limits cell growth. As a matter of fact, air, and especially polluted air, contains some organic substances, but they are usually poorly decomposed and there is not enough to be utilized as food. Besides, there are other unfavourable factors contributing.

Microorganisms contained in air are constantly subjected to drying, which definitely stops all processes. Some bacteria are especially sensitive to water deficits which cause bactericidal effects (e.g. gonococci or spirochete which die as soon as they enter the air). Many organisms, however, can successfully cope with water deficits and, although they cannot function properly, their dried up forms survive months and even years (endospores, fungi spores).

Solar radiation is also damaging to microorganisms suspended in air as it causes mutation and desiccation (in water and soil the solar radiation is usually very weak or simply does not exist) (see also 3.2 and 3.5).

3.2. Adaptation of microorganisms to the air environment

What types of microorganisms occur in air?

There are 3 main groups of microorganisms that occur in air:
- viruses
- bacteria
- fungi

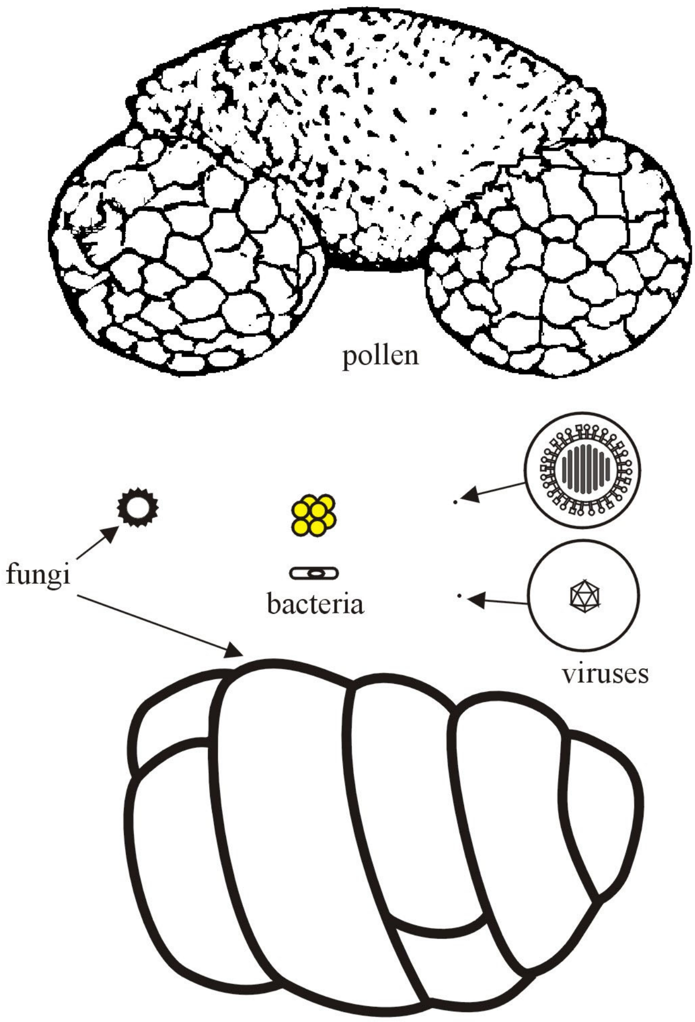

Fig. 3.1. Examples of the biological objects occurring in air
(with the size proportions)

Bacteria may exist as vegetative or resting forms, however fungi occur in the form of spores or fragments of mycelium.

Especially in the vegetative season, pollen of anemophilous plants (e.g. grasses and some trees) is abundant in the air (Fig. 3.1). Besides the above, the following can be found in air as well: algae and protozoa cysts and small invertebrates such as worms in forms of eggs or cysts and mites.

Besides living microorganisms, their fragments and products, which often exhibit toxic or allergic activities, may also occur in air (see 3.6).

Which microorganisms are best adapted to a prolonged existence in air?

The atmosphere can be occupied for the longest time by those forms which, due to their chemical composition or structure, are resistant to desiccation and solar radiation. They can be subdivided into the following groups:

- bacterial resting forms,
- bacterial vegetative forms which produce carotenoidal dyes or special protective layers (capsules, special structure of cell wall),
- spores of fungi,
- viruses with envelopes

Resting forms of bacteria

Endospores are the best known resting forms. These structures evolve within cells and are covered by a thick multi-layer casing. Consequently, endospores are unusually resistant to most unfavourable environment conditions and are able to survive virtually endlessly in the conditions provided by the atmospheric air. They are only produced by some bacteria, mainly by *Bacillus* and *Clostridium* genera. Because each cell produces only one endospore, these spore forms cannot be used for reproduction.

Another type of resting form is produced by very common soil bacteria, the actinomycetes. Their special vertical, filiform cells, of the so-called air mycelium, undergo fragmentation producing numerous ball-shaped formations. Due to the fact that their production is similar to the formation of fungal, they are also called conidia. Contrary to endospores, the conidia are used for reproduction.

There are also other bacterial resting forms, among others, the cysts produced by azotobacters - soil bacteria capable of molecular nitrogen assimilation.

Resistant vegetative cells of bacteria

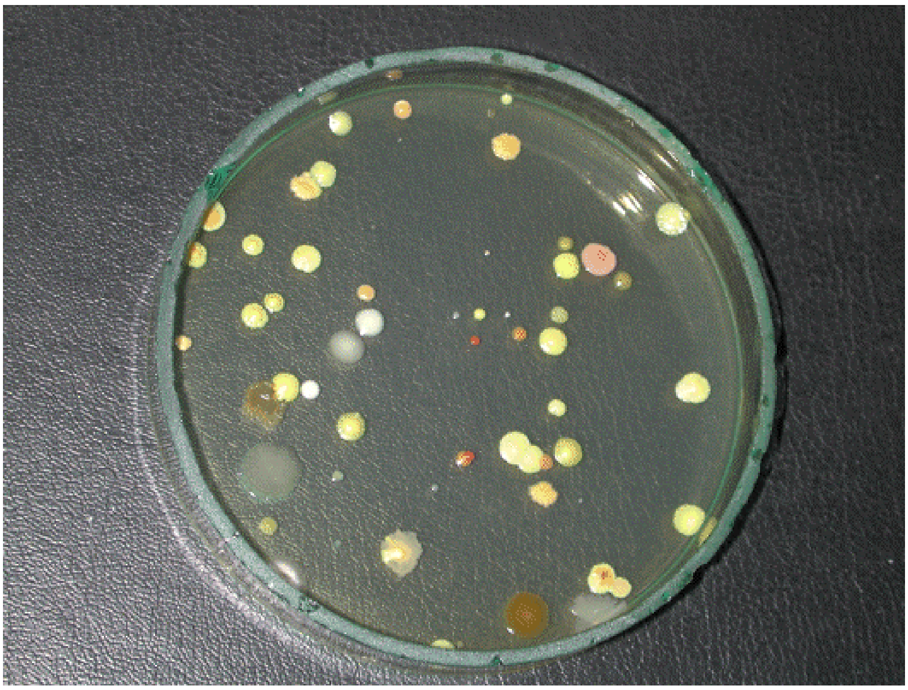

Fig. 3.2. Coloured colonies of airborne bacteria on a Petri plate with agar medium

The production of carotenoidal dyes ensures cells with solar radiation protection. **Carotenoids**, due to the presence of numerous double bonds within a molecule (-C=C-), serve a purpose as antioxidants, because, as strong reducing agents, they are oxidized by free radicals. Consequently, important biological macromolecules are being protected against oxidation (DNA, proteins etc.). Bacteria devoid of these dyes quickly perish due to the photodynamic effect of photooxidation. That explains why the colonies of bacteria, which settle upon open agar plates, are often coloured (Fig. 3.2). The ability to produce carotenoids is possessed especially by cocci and rod-shaped actinomycetes.

Rod-shaped actinomycetes, e.g. *Mycobacterium tuberculosis*, besides being resistant to light, also demonstrate significant resistance to drying due to a high content of lipids within their cell wall. High survival rates in air are also a characteristic for the bacteria which possess a capsule, e.g. *Klebsiella* genus, that cause respiratory system illnesses.

Fungal spores

Spores are special reproductive cells used for asexual reproduction. Fungi produce spores in astronomical quantities, for example the giant puffball (*Calvatia gigantea*) produces 20,000,000,000,000 (20 billion!) spores, which get into the air and are dispersed over vast areas. A very common type of spores found in air is that of conidia.

Conidia (*gr*. *konia* - dust) are a type of spore formed by asexual reproduction. They form in the end-sections of vertical hyphae called conidiophores and are dispersed by wind. The spores of common mould fungi such as *Penicillium* and *Aspergillus* are examples of the above. Spore plants such as ferns, horsetails and lycopods also produce spores. Plant pollen is also a kind of spores.

Resistant viruses

Besides cells, the air is also occupied by viruses. Among those that demonstrate the highest resistance are those with enveloped nucleocapsids, such as influenza viruses. Among viruses without enveloped nucleocapsids, enteroviruses demonstrate a relatively high resistance.

Of course, besides the previously mentioned resistant forms, the air is also occupied by more sensitive cells and viruses, but their survival is much shorter. It is believed, that among vegetative forms, gram-positive bacteria demonstrate greater resistance than gram-negative bacteria (especially for desiccation), mainly due to the thickness of their cell wall. Viruses are usually more resistant than bacteria.

3.3. Biological aerosols

Microorganisms suspended in air as a colloidal system

Microorganisms in air occur in a form of colloidal system or the so-called **bioaerosol**. Every colloid is a system where, inside its dispersion medium, particles of dispersed phase occur whose size is halfway between molecules and particles visible with the naked eye. In the case of biological aerosols, it's the air (or other gases) that has the function of the dispersion medium, whereas microorganisms are its dispersed phase. However, it is quite rare to have microbes independently occurring in air. Usually, they are bound with dust particles or liquid droplets (water, saliva etc.), thus the particles of the bioaerosol often exceed microorganisms in size and may occur in two phases:

- dust phase (e.g. bacterial dust) or
- droplet phase (e.g. formed as the result of water-vapour condensation or during sneezing).

The dust particles are usually larger than the droplets and they settle faster. The difference in their ability to penetrate the respiratory tract is dependent on the size of the particles; particles of the droplet phase can reach the alveoli, but dust particles are usually retained in the upper respiratory tract. The number of microorganisms associated with one dust particle is greater than in the droplet phase.

The size of bioaerosols

The average size of bioaerosols ranges from about 0.02 μm to 100 μm. The sizes of certain particles may change under the influence of outside factors (mainly humidity and temperature) or as a result of larger aggregates forming. By using size criterion, the biological aerosol can be subdivided into the following:

- fine particles (less than 1μm) and
- coarse particles (more than 1μm)

Fine particles are mainly viruses, endospores and cell fragments. They possess hygroscopic properties and make-up the so-called nucleus of condensation of water vapour. At high humidity water collects around these particles creating a droplet phase. Then, the diameter of the particles increases. Coarse particles consist mainly of bacteria and fungi, usually associated with dust particles or with water droplets.

3.4 Mechanisms protecting lungs against bioaerosols

There are two basic mechanisms which remove aerosols from the inhaled air:

- mucous-ciliary apparatus,
- Phagocytosis of lung macrophages

Human and animal respiratory tracts are lined with a multi-row epithelium. The tissue is made up of cylindrical cells equipped with cilia and the so-called goblet cells which produce mucus that covers the entire epithelium. The mucus has a high viscosity due to a high content of mucin, as well as bactericidal properties given by **lysozyme** - an enzyme that hydrolyses cell walls of gram-positive bacteria.

Both cell types make up a functional unity creating the mucous-ciliary apparatus First, the particles contained in air stick to the mucus, then they are swept along the mucus toward the nasal cavity, next they are either excreted with saliva (e.g. during coughing) or swallowed. This mechanism is rather effective with larger particles of coarse bioaerosol.

Fine bioaerosols often escape this trapping and find their way into the pulmonary alveoli. Then, they may get absorbed by the macrophages, which are capable of phagocytosis.

Besides the above, there are also other protective mechanisms, e.g. filtration of larger aerosol particles by hair in the nose, the cough reflex, or the inhibitory action of the natural microflora of the respiratory-tracts mucous membrane - called bacterial interference.

However, the efficiency of these mechanisms isn't always sufficient and, especially at high concentrations of bioaerosol and high invasiveness of microorganisms, the respiratory tracts may get invaded causing pathological changes. In addition, the microorganisms which make up the bioaerosol, may find their way into the organism through a food chain (e.g. contaminated surfaces) or through the skin.

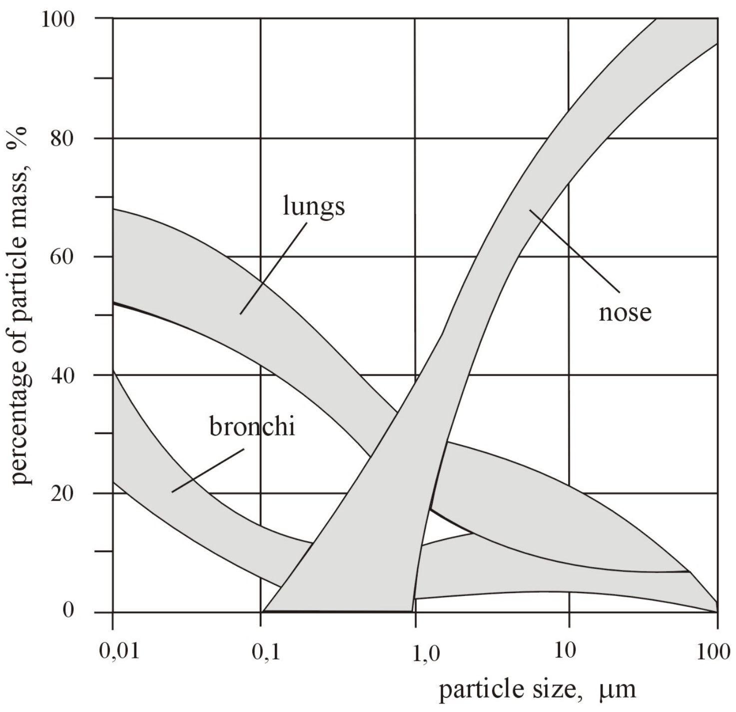

Fig. 3.3. Influence of bioaerosol particle size
on penetration to respiratory tracts (according to Warych, 1999)

Settling of bioaerosols in various parts of the respiratory system is based on the particle size and the strength with which they are inhaled (Fig. 3.3). The same rule governs the non-biological aerosol (dust, smoke, fog).

Coarse grain bioaerosol settles mainly in the nasal and throat cavity (especially particles with a diameter larger than 10 μm) and in the bronchial tubes (particles with a diameter 2-10μm). However, the fine-grained bioaerosol reaches even further, all the way to the pulmonary alveoli (particles with a diameter 1μm and less).

During forceful inhalations (e.g. hyperventilation, cough) the lungs may be reached by larger particles as well (diameter of 10μm. and more). Particles capable of penetrating the pulmonary alveoli are referred to as the **respirable fraction**. This name refers to all types of particles, not only those of the bioaerosol. It is commonly believed that the respirable fractions consist of particles of diameters less than 10μm.

The contribution of the respirable fraction to the bioaerosol is a measure of its harmfulness as it illustrates the part of the bioaerosol that may penetrate the lungs. That is why methods to determine the size of the respirable fractions are most crucial when determining the level of microbiological air pollution.

3.5. Survival and spread of the bioaerosols

Once microorganisms find their way into air, they are influenced by a series of unfavourable conditions, thus a significant number of them perish, mainly as a result of dehydration. The ones that manage to overcome the shock of sudden condition change still continue to be affected by these changes and consequently may die. There are several factors which influence the ability of a bioaerosol to survive in air:

- particular resistance for a given microorganism (*morphological characteristics),*
- meteorological conditions (inter alia, air humidity, solar radiation),
- air pollution,
- the length of time in air.

The kinetics of bioaerosol decay has been illustrated by the following formula:

$$x_t = x_0^{-kt}$$

where X_t represents the concentration of microorganisms after a length of time *t* in air, X_o - the initial concentration, and *k* - decay constant. The constant is specific for a particular type of microorganism and the habitat conditions in which the bioaerosol occurs. The constant is determined experimentally. The given formula illustrates a typical process of the first order, which is characteristic for so many phenomena in nature.
A microorganisms' ability to survive in air may also be expressed by the **time of half-decay $t_{1/2}$,** e.g. for many *Enterobacteriaceae* bacteria that time is 8 seconds.

Resistance of microorganisms

It is a species dependent feature, which relies on the microorganism's morphology and physiology. This is thoroughly discussed in 3.2.

Relative humidity

The content of water in air is one of the major factors determining the ability to survive. At a very low humidity and high temperature cells face dehydration, whereas high humidity may give cells protection against the solar radiation.

Microorganisms react differently to humidity variations in air, but nevertheless most of them prefer high humidity. The morphology and biochemistry of cell-surrounding structures, which may change its conformation depending on the amount of water in air, are crucial. Actually, an exact mechanism of this is not known. Forms of resting spores with thick envelopes (e.g. bacterial endospores) are not particularly susceptible to humidity variations. Gram-negative bacteria and enveloped viruses (e.g. influenza virus, myxo) deal better with low air humidity which is contrary to gram-positive bacteria and non-enveloped viruses (e.g. enteroviruses) that have higher survival rates in high air humidity.

Temperature

Temperature can indirectly affect cells by changing the relative-air humidity (the higher the temperature, the lower the relative humidity) or a direct affect, causing, in some extreme situations, cell dehydration and protein denaturation (high temperatures) or crystallization of water contained within cells (temperatures below 0°C). Therefore, it can be concluded that low (but above 0°C) temperatures are optimal for the bioaerosol (according to some researchers the optimal temperatures are above 15°C).

Solar radiation

Solar radiation has a negative affect on the survival rate of the bioaerosol, both visible as well as ultraviolet (UV) and infrared radiation due to the following factors:

- it causes mutation,
- leads to the formation of free radicals, which damage important macromolecules.
- creates a danger of dehydration.

Visible-light rays of about 400-700 nm wavelength, create the so-called photodynamic effect, which produces free radicals within cells, especially compounds such as peroxy and hydroxyl radicals. These radicals demonstrate strong oxidizing activities and may cause damage to crucial macromolecules, e.g. DNA or proteins.

UV radiation has a much larger affect on cells than visible light does, especially the rays of 230-275 nm wavelength. The mechanism of this effect is based on changes to DNA, both directly (e.g. by creating thymine dimer and consequently causing mutation), as well as indirectly, by creating free radicals as in the case of the visible light.

In addition, infrared (IR) radiation may have a negative effect upon cells contained in air - heating up and consequently dehydration.

Air pollution

Pollutants in air, especially hydrocarbons, ozone and nitric oxides, which are activated by solar radiation (especially UV) create various, highly reactive secondary pollutants, commonly described as photochemical oxidizers (among others peroxiacetyl nitrate - PAN and organic peroxides). They are toxic to all living forms including air-suspended microorganisms.

In contrast, the non-toxic and non-biological aerosols (dust, fog), disperse and absorb the solar radiation which consequently increases the survival of bioaerosols.

It should be noted that the above factors often work simultaneously and are related, e.g. solar radiation increases the temperature, whereas high humidity weakens the radiation.

What does the concentration of the bioaerosol depend on?

The concentration of bioaerosol is dependent on the following factors:
- amount of emitted microorganisms, depending on the emitter,
- distance from the source of emission,
- wind speed,
- microorganisms' survival rate, depending on the factors discussed above,
- precipitation.

The amount of emission and quality content of the emitted bioaerosol depends on the source of the emission. There can be different factors that affect the initial concentration of the bioaerosol. For example, for the aeration tank of a biological treatment plant the factors are: the concentration of microorganisms in sewage and the method of aeration. The concentration has to be adequately high, otherwise bioaerosols won't be created. It can be described by the so-called emission threshold, which is equal to 10^3 cells per 1 cm^3 for sewage.

The newly created bioaerosol disperses in a similar way to the non-biological one (e.g. suspended dust) except that the microorganisms die out with time. Blowing wind dilutes the aerosol and causes its concentration to drop as it moves away from the place of emission. In addition, a concentration decrease is caused by gravitational sedimentation (acting on the larger particles) and as a result of precipitation.

3.6. Biological aerosols as a human hazard source

What types of dangers are connected to the presence of microorganisms in air?

- Infectious diseases (viral, bacterial, fungal and protozoan),
- Allergic diseases,
- Poisoning (exotoxins, endotoxins, mycotoxins).

Bioaerosols may carry microorganisms other than those which evoke respiratory system diseases. The intestinal microorganisms contained in aerosols may, after settling down, get into the digestive system (e.g. by hands) causing various intestinal illnesses.

Infectious airborne diseases

The mucous membrane of the respiratory system is a specific type of a 'gateway' for most airborne pathogenic microorganisms. Susceptibility to infections is increased by dust and gaseous air-pollution, e.g. SO_2 reacts with water that is present in the respiratory system, creating H_2SO_4, which irritates the layer of mucous. Consequently, in areas of heavy air pollution, especially during smog, there is an increased rate of respiratory diseases.

Bioaerosols may, among other things, carry microbes that penetrate organs via the respiratory system. After settling, microbes from the air, may find their way onto the skin or, carried by hands, get into the digestive system (from there, carried by blood, to other systems, e.g. the nervous system). Fungi that cause skin infections, intestinal bacteria that cause digestive system diseases or nervous system attacking enteroviruses are all examples of the above.

Viral diseases

After penetrating the respiratory system with inhaled air, particles of viruses reproduce inside the cuticle cells of both the upper and lower respiratory system. After reproduction some of the viruses stay inside the respiratory system causing various ailments (runny nose, colds, bronchitis, pneumonia), whereas others leave the respiratory system to attack other organs (e.g. chickenpox viruses attack the skin). The most noteworthy viruses are:

- influenza (orthomyxoviruses)
- influenza, measles, bronchitis, mumps and pneumonia among newborns (paramyxoviruses)
- German measles (similar to paramyxoviruses)
- colds (rhinoviruses and koronaviruses)
- cowpox and true pox (pox type viruses)
- chickenpox (cold sore group of viruses)
- foot-and-mouth disease (picorna type viruses)
- meningitis, pleurodynia (enteroviruses)
- sore throat, pneumonia (adenoviruses)

Bacterial diseases

Similarly to viruses, some bacteria that find their way to the respiratory system may also cause ailments of other systems. Especially staphylococcus infections assume various clinical forms (bone marrow inflammation, skin necrosis, intestinal inflammation, pneumonia). Often, a susceptible base for development of various bacterial diseases is first prepared by viral diseases, e.g. staphylococcus pneumonia is usually preceded by a flu or mumps. Bacterial airborne diseases include:

- tuberculosis (*Mycobacterium tuberculosis),*
- pneumonia (staphylococcus, pneumococci, *Streptococcus pneumoniae,* less frequently chromatobars of *Klebsiella pneumoniae),*
- angina, scarlet fever, laryngitis (streptococcus)*,*

- inflammation of upper and lower respiratory system and meningitis (*Haemophilus influenzae),*
- whooping cough (chromatobars of *Bordetella pertussis*),
- diphtheria (*Corynebacterium diphtheriae),*
- legionnaires disease (chromatobars of *Legionella* genus, among others *L. pneumophila),*
- nocardiosis (oxygen actinomycetes of *Nocardia* genus).

Fungal diseases

Many potentially pathogenic airborne fungi or the so-called saprophytes live in soil. They usually have an ability to break down keratin (keratinolysis) - difficult to decompose proteins found in horny skin formations, e.g. human or animal hair, feathers, claws. Some of the **keratinolytic fungi,** the so-called **dermatophytes**, cause **mycosis of the outer skin** (dermatosis)**,** as the break down of keratin enables them to penetrate the epidermis. Other fungi, after penetrating the respiratory system, cause **deep mycosis** (organ), e.g. attacking lungs. The following are examples of airborne fungi diseases:

- mycosis (*Microsporum racemosum),*
- deep mycosis: aspergillosis (*Aspergillus fumigatus)*, cryptococcus (*Cryptococcus neoformans*).

Protozoan diseases

Some protozoa, which are able to produce cysts that are resistant to dehydration and solar radiation, may also infect humans by inhalation. The most common example of the above is: *Pneumocystis carinii* which causes pneumonia.

Dangers connected with pathogenic bioaerosols do not concern only human diseases. Other significant diseases are those that attack cultivated plants or farm animals. The following are examples of the above:

- blight - grain disease caused by *Puccinia graminis,* and
- aphthous fever - very infectious disease that attacks artiodactylous animals.

Allergic diseases

Allergy is a changed, hypersensitive reaction of the person or animal to some substances called allergens (gr. *allos* - other, *ergon* - action). Actually, it's an immunologic reaction, in which a needless production of antibodies by B lymphocytes (mainly IgE and IgG immunoglobulins) occurs as a hypersensitive response to penetration of antigens (called the **allergen**). Excessively produced immunoglobulins combine with allergens, which cause among other things:

- a release of various compounds (e.g. histamines) from mast cells.
  The released compounds induce inflamed reactions in the form of bronchus asthma or hay fever,
- cause damaged tissue at the place of contact, - allergic pulmonary alveoli inflammation (e.g. the so-called farmer's lung, or mushroom breeder's lung).

Many microbes exist as allergens. Besides these, there are other allergenic factors such as anemophilous pollens (e.g. grass, nettle, comose), small arachnids (mites) as well as biological dust (e.g. particles of feathers, hair or droppings). Microorganisms differ in their allergenic influences. The strongest allergens are mold fungi, thermophilus actinomycetes, as well as Gram-negative chromatobars. The strength of allergenic bioaerosols depends not only on the type of microorganisms but also on their concentration.

A type of allergic reaction induced by biological aerosols depends on the type of allergens that cause it as well as, to a large extent, the size of its particles as it determines the degree of penetration into the respiratory system:

- particles larger than 10μm, held in the nasal cavity, cause hay fever (e.g. fungi spores of *Alternaria,* grass pollen) (Fig. 3.4),
- particles of diameter between 4-10μm, held in bronchi, cause asthma (e.g. fungi spores of *Cladosporium*) (Fig. 3.5),
- particles larger than 4μm, that penetrate alveoli, besides asthma, induce allergic inflammation of pulmonary alveoli (fungi spores of *Aspergillus* and *Penicillium*).

Fig. 3.4. *Alternaria* sp. – spores

Fig. 3.5. *Cladosporium*. sp. – spores.

Poisoning

Poisoning / intoxication is caused by toxins that are produced by some microorganisms. Endotoxins and mycotoxins are the most significant types of toxins in polluted air.

Endotoxins are the components of Gram-negative bacterial cell walls (A lipid fragment of lipopolysaccharides LPS outer membrane) (Fig. 3.6). They demonstrate toxic (and allergenic) effects on mammals. After being inhaled into the lungs, they cause acute inflammation of the lungs.

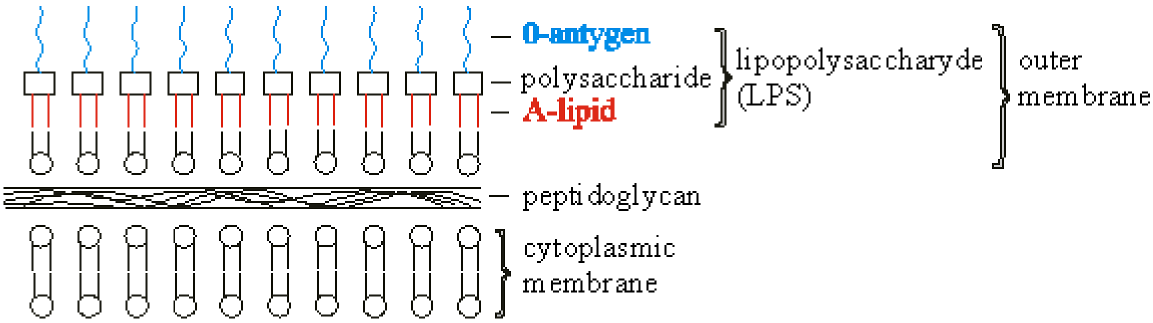

Fig. 3.6. Localisation of endotoxin in Gram-negative bacterial cell wall.

Mycotoxins are produced by various mold fungi. The most common ones are **aflatoxins** produced by *Aspergillus flavus.* These compounds (there are several types of them) demonstrate strong toxic, mutagenic, carcinogenic, teratogenic (cause malformation in a fetus) actions. Most often they lead to food poisonings, however it has also been indicated, that inhaling dusts which contain aflatoxins may bring about tumours of the liver and the respiratory system.

3.7. Basic sources of bioaerosol emission

There are two basic **sources of bioaerosol**:

- natural,
- related to human activities

Natural sources are mainly soil and water, from which microorganisms are being lifted up by the movement of air, and from organisms such as fungi, that produce gigantic amounts of spores that are dispersed by the wind.

Therefore, there are always a given number of microorganisms in the air, as a natural background. It is estimated, that the air is considered to be clean, if the concentration of bacteria and fungi cells does not exceed $1000/m^3$ and $3000/m^3$ respectively. This latter statement is only true when the concentration of microorganisms consists of saprophytic organisms, not pathogenic organisms. If the concentration of microorganisms in the air exceeds the above values, or contains microorganisms dangerous to humans, then such air is considered to be **microbiologically polluted.**

From the hygienic point of view, **living sources of bioaerosols** related to human activity, are more important than the natural sources. The emissions from these sources are dangerous due to the following two reasons:

- they may distribute pathogenic microorganisms,
- they often cause a high increase of microorganisms in the air, significantly exceeding the natural background.

The emission sources of biological aerosols can have a localized character (e.g. aeration tank) or a surface character (e.g. sewage-irrigated field).
The most important sources of bioaerosol emission are:

- agriculture and farming-food industry,
- sewage treatment plants,
- waste management.

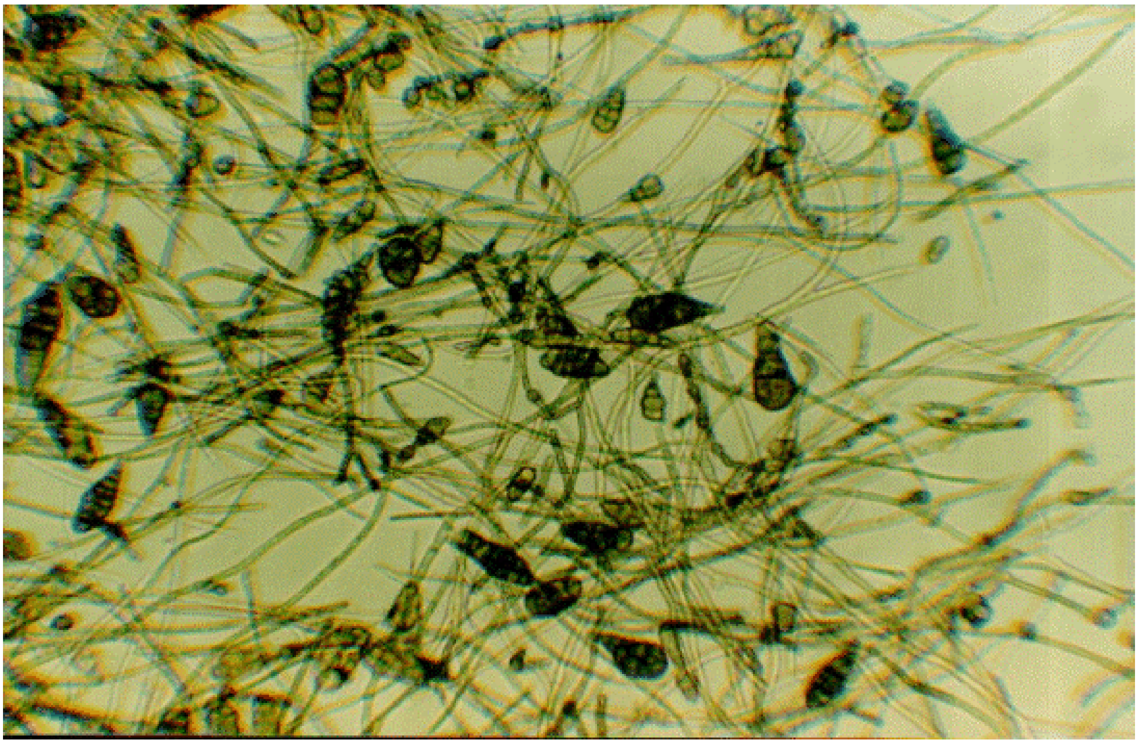

Fig. 3.7. *Alternaria sp.*-mycelium and spores

3.7.1. Agriculture and food/farming industry

This is the biggest source of bioaerosol emission, which results from the intensification of methods of farm production. Aerosols are created during almost all types of agricultural work, e.g. harvest, transport, storing, plant and animal material processing, and in animal breeding facilities.

The significant dangers to human health are brought about by a vast number of microorganisms, products of their decomposition and of organic dust, which have an allergic and toxic affect. The presence of infectious microbes is less significant here. The most important components of the above-mentioned aerosols are:

- mold fungus, *Aspergillus, Penicillium* and *Cladosporium, Alternaria* (Fig. 3.7),
- Gram-negative chromatobars, mainly of the genus *Erwinia,*
- thermophilic acitnomycetes,
- dust of biological origin (among other things: particles of skin, feathers, droppings, plant dust).

Contact with such aerosols may often bring about chronic diseases of the respiratory system, e.g. allergic inflammation of pulmonary alveoli (*Alveolitis allergica).* It is said, that there is an exceptional health risk, when over 50% of the aerosol belongs to the respirable fraction, and bioaerosol's concentration exceeds 10^5 cfu/m^3. The above quantity is often exceeded a hundred times (e.g. in pigsties, broiler houses, granaries).

3.7.2. Wastewater treatment

The size of bioaerosol emission depends on - among other factors - the make-up of sewage, sewage-treatment-plant flow capacity, as well as the methods and types of the equipment. Favourable conditions for bioaerosol formation are at the time of sewage spouting, aeration, mixing and dispersing. The quality of the air-microflora make-up is closely connected to the content of the treated sewage.

In mechanical-biological sewage treatment plants, which utilize the activated-sludge method, the biggest emission of microorganisms can be observed in the mechanical section, where raw sewage is introduced (drainage area, grill, sand trap) as well as in the vicinity of aeration tanks and sludge drying beds. Moreover, a strong emission may be also observed during sewage purification in soil and in sprinkling beds. Significant air pollution may occur in the areas stated above, which may even exceed the level of background concentration. The following are some of the characteristic microorganisms present in bioaerosol and sewage:

- intestinal bacteria (*Enterobacteriaceae),* including those of the *coli* group, *Salmonella, Shigella,*
- hemolysis bacteria, mainly streptococcus and staphylococcus,
- *Pseudomonas* bacteria,
- yeast such as *Candida albicans and Cryptococcus,*
- dermatophites (surface-mycosis inducing fungus) of *Microsporum and Trichophyton* genus,
- protozoa,
- eggs of worms,
- intestinal viruses: enteroviruses and reoviruses.

From the stated microorganisms, the intestinal bacteria and viruses are most specific to sewage bioaerosols, and usually don't occur in the down-wind side of the plant. Consequently, they are considered to be indicator microorganisms that are helpful in determining the effect of the sewage-treatment plant upon the surrounding environment. In addition, the air in the plant's facility also contains endotoxins, in some cases at concentrations exceeding the maximum limit.

Bioaerosols that originate in biological sewage-treatment plants usually do not disperse more than a few hundred meters; actually, the pollution level is already much lower

50 meters away from the source of the emission. Thus, they are dangerous, mainly to those, who are directly within the facility. Blood tests of workers who are subject to aerosol inhalation indicated an increased level of antibodies of gram-negative bacteria and intestinal viruses. The condition has been described as 'the sewage worker's syndrome', which has a viral origin and manifests itself with a despondency, overall weakness, catarrh and fever. Moreover, sewage workers and those who live in the vicinity of a treatment plant have higher morbidity with intestinal and respiratory system illnesses.

3.7.3. Waste management

Various forms of waste management are additional sources of bioaerosol emission:
- waste storage and
- composting.

Waste storage

The air around storage yards contains bacteria found commonly in nature and saprophytic fungi of soil and water origin, some of which are opportunistic pathogens. It means that under favourable conditions (weakening of the defence system, penetrating the body in large numbers) they may invoke various diseases in humans. The following are the dominating genera of bacteria: *Bacillus, Pseudomonas, Enterobacter.* The last two genera are Gram-negative bacteria which produce endotoxins and their presence is often observed around waste dumps. In close proximity to dumps, the concentration of microorganisms often exceeds $10^5/m^3$ (Fig. 3.8). It is believed that within dump sites and similar communal facilities (e.g. composting site), the total number of bacteria in air should not exceed $10^4/m^3$, and of Gram-negative bacteria $10^3/m^3$.

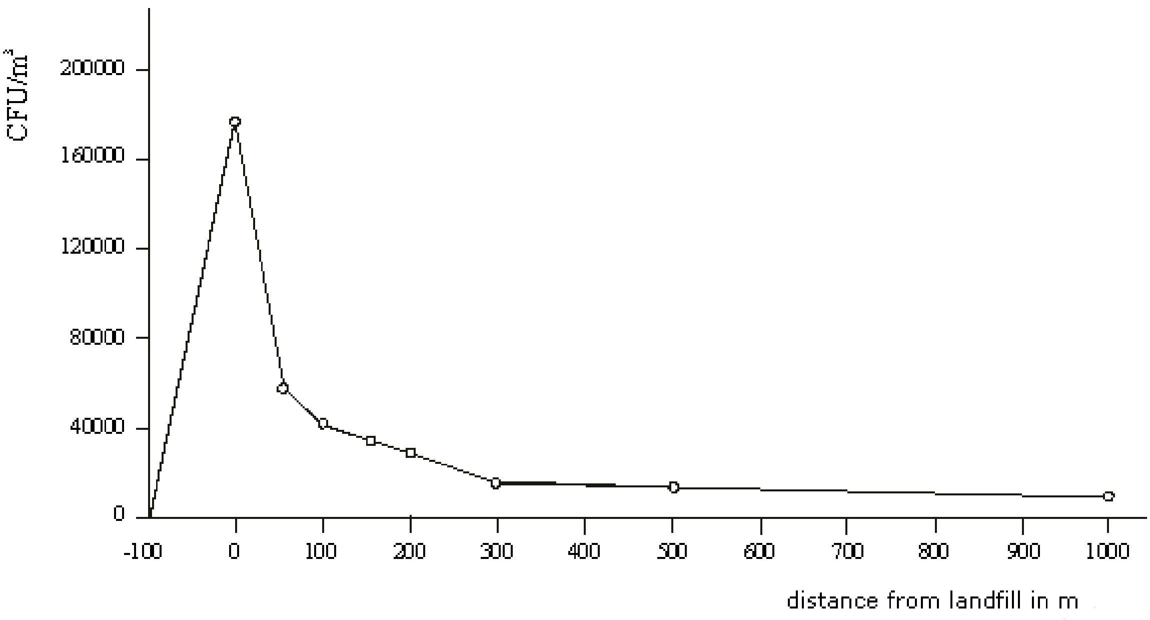

Fig. 3.8. Bioaerosol concentration near the municipal landfill site (according to Kulig, Ossowska-Cypryk, 1999)

Thermophilic (flourish in temperatures at 37°C) and keratinolytic (breaking down keratin) fungi are favorable indicator microorganisms of a dump's impact upon the surroundings. Waste dumps provide suitable conditions for development of these types of fungi due to increased humidity, temperature and numerous particles containing keratin - proteins which are difficult to decompose found among other places, in hair, feathers, claws.

The thermophilic species of mold fungus (*Alternaria alternata, Mucor pusillus, Aspergillus ochraceus)* and yeast-like fungi (*Candida sp.* and *Geotrichum candidum)* are quite common. They may induce allergies and produce mycotoxins (especially molds) as well as cause deep mycosis (e.g. in respiratory system).

Keratinolytic fungi are typical soil microorganisms that include numerous dermatophytes (mycosis inducing fungus, e.g. *Microsporum racemosum).* The ability to assimilate keratin allows them to penetrate the skin layers and hair.

The range of bioaerosols spread by waste storage facilities is usually greater than that of sewage-treatment plants and may often exceed 1000 meters.

Composting

Composting also emits large amounts of microorganisms - especially bacteria. Particularly large air pollution is created during waste sorting, when the concentration of bacteria often exceeds the limit of 10^5 CFU/m^3.

Among these, there are, Gram-negative bacteria which are potentially harmful to humans. Due to high temperatures (65-70°C) of the composting process most of the above bacteria usually get neutralized, however their endotoxins demonstrate a certain degree of thermostability thus, when released into air, they can cause poisoning.

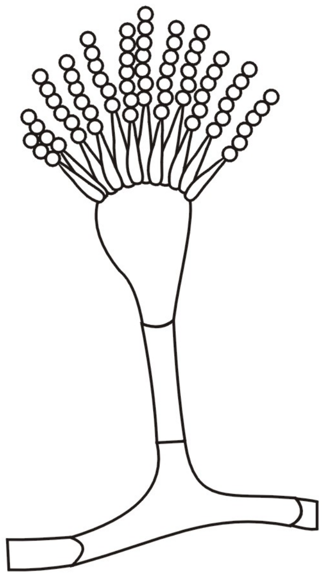

Fig. 3.9. *Aspergillus fumigatus*
– the indicator organism for a composting plant

The common mold fungus, *Aspergillus fumigatus* (Fig. 3.9)*,* whose spore concentration in air can equal 10^6/m^3, is a good indicator of the effect of the composting facilities on its surroundings. The above species is a typical opportunistic pathogen which, among other diseases, causes aspergillosis and allergic diseases of the respiratory system (e.g. A*lveolitis allergica*).

3.8. Investigation of microbiological air pollutions

3.8.1. Detecting the presence of microbes in air

Methods utilized can be subdivided into:

- microscopic and
- culture

Sometimes the methods are a combination of both.

Microscopic methods

They consist of:

- letting air through a membrane filter or placing a glass coated with a sticky substance (e.g. vaseline), in the air's path
- staining of the trapped microorganisms and
- microscopic testing consisting of cell counting.

Staining with acridine orange and examination under a fluorescence microscope is often applied. The final result is given as a total number of microbes in 1 m^3 of air.

The advantage of the above methods is that it allows the detection of live and dead microbes in air, as well as those, which do not abundantly flourish in culture media. Due to this, the number of microbes determined is usually higher by one order of magnitude than in culture methods. In addition, it is possible to detect and identify other biological objects, e.g.: plant pollen, allergenic mites, abiotic organic dust (fragments of skin, feathers, plants, etc.).

However the methods have a serious drawback: inability to determine the species of microbes (bacteria, fungi, viruses).

Culture methods

These methods consist of transferring microbes from air onto the surface of the appropriate culture medium. After a period of incubation at optimal temperature, the formed colonies are counted and the result is given as **cfu/m^3** of air (colony forming units). Because a colony can form not only from a single cell, but also from a cluster of cells, the air may contain more microbes than suggested by the CFU result. Besides, the method allows the detection of only the cells that are viable and those which are able to grow upon the medium used.

Microbes transferred to the culture medium require resuscitation as they were subjected to the influence of unfavourable conditions. Therefore it is recommended to supplement the culture mediums with such components as betaine and catalase. Betaine, the methylic derivative of the glycine amino acid, is utilized by bacteria to maintain osmotic balance, and as a donor of methylic groups it is essential during the processes of biosynthesis. Catalase however breaks down harmful peroxides created in air as a result of UV radiation.

Testing of viruses differs significantly from the methods utilized for other organisms because:

- they may develop only in living cells, therefore they require tissue cultures (e.g. the epithelium of human trachea or monkey's kidney) or, in the case of bacteriophages, bacterial cultures,
- species identification of detected viruses is meticulous and, among other things, consists of performing electrophoresis or utilizing antiserum that contains antibodies of common viruses,

- drawing large quantities of air is essential (over 1000 dm^3, at least one order of magnitude higher than in the case of bacteria), as the amount of viruses in air is rather small (this especially concerns the enteroviruses).

After transferring the viruses onto the surface of a single-layer culture, the viruses penetrate the cells, reproduce in them, and after their destruction attack the neighboring cells. Consequently, the areas around the initial places of the cell infections get cleared of cells – this clearing is called **plaques.** Therefore, the number of viruses detected is given as the number of units that form the plaques, in short **pfu/m³** (plaque forming units). It has to be pointed out though, that such a method only allows the detection of viruses capable of infecting the utilized cells.

There are three basic ways of sampling the air for use in culture methods:
- Koch's sedimentation method,
- filtration method (also used in microscopic methods),
- impact methods.

Sedimentation method

It is the simplest method that consists of cell settling from the air onto the open Petri dishes filled with the appropriate culture media. The gravitational force affecting the particles of the bioaerosol have significance only in relation to bigger particles, as the smaller ones hit the exposed culture medium as a result of air movement. After a particular period of exposure (usually 10-30 min) the plate is incubated and the resultant colonies counted.

The benefit of this popular method, besides its simplicity, is its inexpensiveness. However it only gives a rough estimate of the number of microbes in air, as it contains a series of problems. They are:
- no knowledge about the air volume, to which the number of cfu can be related,
- inability to detect the smallest particles of bioaerosol which make up the respiratory fraction, that settle very slowly or never undergo the process of sedimentation (low output),
- high inaccuracy, caused by the movement of air that changes the conditions of sedimentation.

The first problem, stated above, can be partially compensated by utilizing the empirical conversion formula, which is based on the assumption that cells contained in 10 dm^3 of air settle upon a 100 cm^2 area in 5 minutes. The formula is as follows:

$$x = \frac{a \cdot 5 \cdot 10^4}{\pi r^2 \cdot t}$$

where: x – number of microbes in air (w cfu/m^3),
a – number of colonies upon the Petri dish,
pr^2 – surface of Petri dish (w cm^2)
t – time of exposure (minutes).

In order to limit the disturbance connected with the movement of air, it is recommended to conduct the testing in low winds.

Filtration methods

These methods are also rather inexpensive and not complicated, they possess two significant advantages over the sedimentation methods:
- the volume of the air tested is known,
- it is possible to detect the very small aerosol that creates the respiratory fraction (nevertheless it is still impossible to determine its size).

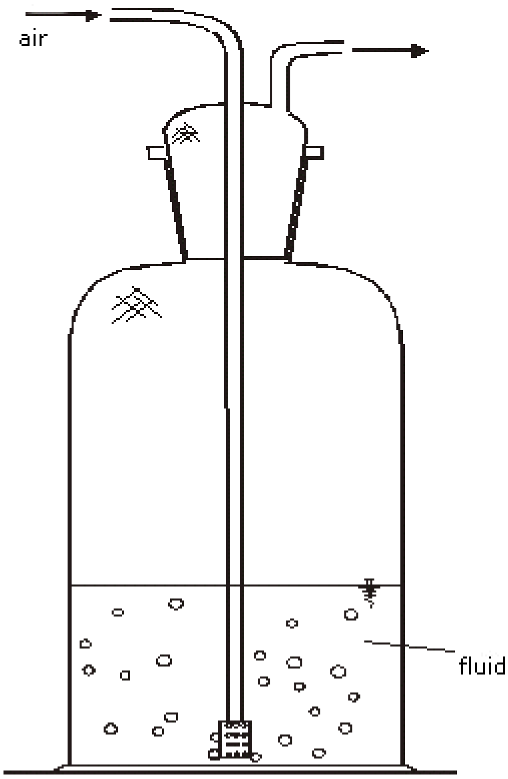

Fig. 3.10. Washer bottle for bioaerosol absorption in filtration methods

The methods consist of using an aspirator to suck in a given volume of air, passing it through a sterile absorbing substance (liquid or solid) and transferring the filtered microbes onto the appropriate culture medium. After a pre-determined time of incubation the resulting colonies are counted. Most often, a membrane filter or a physiological solution (0.85% NaCl) is utilized for the filtration of air.

Filtration using liquids (sometimes classified as the impact method) is one of the most often used and highly valued techniques of sampling bioaerosol (Fig. 3.10).

It results in high output of microbe isolation (including the respirable fraction), as well as significant survival of the filtered microbes. The method may be utilized in virus testing as long as the remaining microbes are neutralized (e.g. with chloroform) and the liquid is concentrated before its introduction into the cell culture.

The filtration process through membrane filters allows the utilization of both culture methods (filters containing microbes are placed directly upon the culture media or are rinsed and then inoculated) as well as the microscopic methods (filters are stained and observed under a microscope). However, the disadvantage of this method is that it has a significantly low output as the process of passing the air through pores of the filter creates resistance. That's why the method is not recommended for microbe testing, but is routinely put to use in detection of endotoxins in air.

Impact methods

These methods consist of using an aspirator to suck in a pre-determined amount (volume) of air, which collides with the nutrient agar at high speed. It causes the microbes in the air to stick to the surface, which after a specific time of incubation, form colonies.

The impact methods are the most highly valued and most often used methods of detecting microbes in air. Their biggest advantage is the possibility of detecting and determining the respiratory fraction of the bioaerosol, in other words, determining the size distribution of its particles. It is very important as the size of particles determines the degree of respiratory system penetration (Fig. 3.3). Moreover, the methods can be utilized to test viruses (trapped microbes are swept from the surface of the culture medium and, after the elimination of other microbes with chloroform, introduced into the cell culture).

A disadvantage for the impact method is a decline in the microbes viability caused by the shock of a sudden collision with nutrient agar and also a possibility of the nutrient culture getting overgrown in cases of high air pollution. The above stated methods are usually not cheap.

The most widely known device that is based on the impact technique is the Andersen's apparatus, in which the air is drawn in passes through six vertically positioned sieves. A Petri dish with nutrient agar is placed underneath each sieve. The speed of the passing air increases as it passes through the consecutive sieves, consequently causing greater impact force as it collides with the sieves. As a result, the heaviest (largest) particles settle upon the first sieve, whereas the lighter (smaller) ones are drawn in by the current of the passing air. As they pass through the consecutive sieves, the increasingly smaller and faster particles collide with the nutrient agar. Consequently the particles of the biological aerosol are sorted according to their size and the colonies are then derived from particles of particular size. This way, by counting the colonies upon the consecutive plates, it is possible to determine the ratio of particles which settle in the upper (higher positioned plates) and lower respiratory system (lower plates).

3.8.2. Detecting toxins and allergens in air

Endotoxins

Detecting endotoxins includes the following stages:

- air filtration through a membrane filter (made of glass fibre or polyvinyl chloride),
- reactions of a series of dilutions of filtered cells with a Limulus lysate blood preparation with an addition of a chromogenic substance,
- measurement of the luminescence formed.

Limulus polyphemus is a marine arthropod related to arachnids, which lives of the shores of North America. It is best known for its peculiar immunologic system. Its blood cells (amebocyte), after coming into contact with cell walls of Gram-negative bacteria (containing endotoxins) release an enzyme that causes coagulation of particular blood proteins and formation of a clot that immobilizes the bacteria.

The activation of the coagulation-by-endotoxin enzyme is a very sensitive reaction. Produced on a large scale Limulus Amebocyte Lysate (LAL) with the addition of a chromogenic substance that demonstrates luminescence in cases of clot formation (in the presence of endotoxins) is widely used in air testing. The measurement of luminescence allows the determination of the amount of endotoxins in the air that is being tested.

Other toxins and allergens

Their detection often requires meticulous testing and is based on:

- immunologic reactions that use antibodies used against well known Antigens (e.g. allergens),
- chromatographic testing (e.g. mycotoxins).

3.8.3. Microbiological evaluation of air pollution

Evaluation of air pollution includes both quantity and quality aspects, and depends on the type of air evaluated. Different criteria are used for atmospheric air and air inside various rooms. The values of safe concentration vary from author to author.

According to the norms assumed in Poland the atmospheric air is clean when the concentration of bacterial cells does not exceed 1000 cfu/m^3 - fungi 3000 cfu/m^3. Of course only when the organisms are saprophytic not pathogenic.
Inside a building the total number of bacteria should not exceed 2000 cfu/m^3 - or fungi 300 cfu/m^3. When the concentration of microbes exceeds the above norms, or when the aerosol contains harmful microbes, then such air is considered to be microbiologically polluted.

Norms for other rooms depend on its pre-determined use, e.g. surgery room may not contain any fungus and the number of bacteria cannot exceed 100 cfu/m^3, whereas in a pigsty it is 200 000cfu/m^3 of bacteria and 10 000 cfu/m^3 of fungi.

Very important from a hygienic point of view is the knowledge about the particle size distribution of the bioaerosol. The greater the proportion of small particles in the bioaerosol, which can enter the alveoli (size about 1μm), the greater the health hazard of the air, even if there are no microorganisms causing infectious diseases in the aerosol. The inhalation of such air may cause allergy, poisoning and dust diseases (see 3.6 and 3.7.2).

Qualitative examinations perforce must be limited to indicator microorganisms (see 3.7.3), as the identification of pathogenic microbes is usually strenuous and expensive. The indicator species need not be pathogenic, but their occurrence points indirectly to a potential threat due to disease-causing microorganisms.

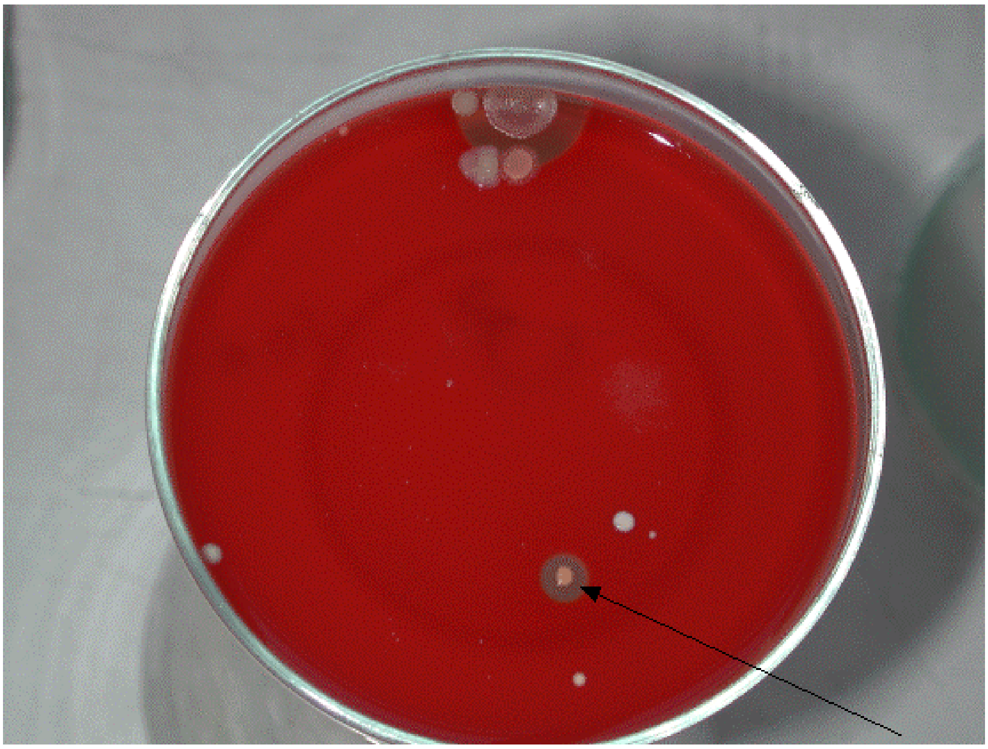

Fig. 3.11. Colonies of bacteria on blood agar medium.
Around one of them (arrow) the zone of hemolysis is visible

The following indicator microorganisms are used for microbial analysis of the air:

- hemolytic staphylococci,
- mannitol fermenting and not-fermenting staphylococci,
- actinomycete bacteria,
- Pseudomonas fluorescens.

Staphylococci are one of the most common bacteria in nature. They are not all pathogenic; many of them appear on human skin and the mucous membranes and do not cause diseases. Pathogenic staphylococci show a high metabolic activity, which can be used to differentiate them from the non-pathogenic ones.

Pathogenic staphylococci cause:

- hemolysis of red blood cells (erythrocytes) on blood agar medium,
- acid fermentation of mannitol on mannitol salt agar medium (Chapman medium).

The **hemolysis** consists of the destruction of erythrocytes by certain toxins produced by bacteria, which results in the formation of a characteristic zone of clearing around the colony (Fig. 3.11).

The Chapman medium contains 10% NaCl, which ensures that mainly staphylococci grow on this medium (they are resistant to high concentrations of salt). The presence of mannitol in the medium is used to differentiate the mannitol-fermenting staphylococci from the non-fermenting ones. Determination of haemolysis and of the fermentation of mannitol increases the probability that staphylococci detected are pathogenic.
Actinomycetes are typical soil bacteria. Their presence in the air can point to the soil environment as the source of the pollution.

Pseudomonas fluorescens is a common water bacterium. Its presence in the air can point to the water environment as the source of pollution.

In addition to the investigation of the microbiological air pollution emitter, one uses typical species for this emission source (3.7). This can determine its impact on the state of pollution of the air – the occurrence of the indicator microorganisms will mark the border of the impact zone.

Evaluation

Why is the survival of microorganisms in air greater at night and on cloudy days, than during sunny days?
Name all morphological forms, in which bacteria and fungi can appear in the air.
Compare the dust phase and droplet phase of the biological aerosol.
State and discuss the recognised defensive mechanisms against bioaerosol penetration to the respiratory system.
Discuss the dependence of size on the penetration of aerosol particles in the respiratory system. Give examples.
State the bacterial and the viral airborne diseases.
What threats can be created by the biological aerosol which doesn't transmit infectious diseases? Discuss one of them.
What is an allergy and which airborne particles are allergens?
State the most important man-made sources of bioaerosol and discuss one of them.
What difficulties are associated with the culturing of airborne microorganisms?
Formulate two critical observations on the manner of the presentation of investigation results in "cfu".
Which of the biological aerosol components cannot be detected with the deposition method and why?
Does the growth of very few colonies on agar medium (or the lack of the growth) indicate that the air is not harmful? Give reasons for your answer.
What are the keratinolytic fungi and why are they used as indicator microorganisms for the examination of the influence of landfills on the state of pollution of the air?
How can one detect the presence of endotoxins in air?
How can one detect viruses in air?
What are indicator microorganisms? Give examples.

Summary

Microorganisms in air are subjected to many limiting factors which inhibit their development. Solar radiation and low relative humidity cause cell desiccation and are the most important limiting factors. Longer survival in air is possible only for resting forms or for cells producing carotenoids which act as antioxidants. Microbes create a biological aerosol in air which spreads similarly to particulate pollutants. The penetration in the respiratory system by bioaerosol particles depends on their sizes. The smallest particles (about the diameter of 1 μm or less) penetrate deeper into terminal bronchioles and alveoli and are the most dangerous to health. Threats connected with the biological aerosol are not limited only to the spread of the microorganisms causing infectious diseases. A serious threat is caused also by organic dusts containing numerous allergens and toxins, including endotoxins produced by gram-negative bacteria (e.g. the dust nascent during agricultural work).
The research methods used for examination of microbiological pollutants of air can be divided into microscopic and culture methods. The most important are these methods which enable the discrimination of the particle size. The indicator organisms for a particular emitter play an important role in the investigation of the influence of the definite emission source on the health risk of the air.

Glossary

Antigen – the protein or polysaccharide which after penetration into a cell can stimulate it to produce antibodies – the proteins (immunoglobulins) which selectively connect with the antigen and inactivate them (antibodies are produced by lymphocyte cells). The microorganisms, and specifically their surface structures, e.g. the O-antigens and endotoxins building the cell wall of gram-negative bacteria, are antigens. Specific antigens are allergens. The name "antigen" is shortened from the Latin *anticorporis generator* that is literally "the producer of antibodies".

Biocenosis (microbiocenosis) – the association of organisms (of microorganisms) which reside in a common biotop (the living environment), between which the various interactions are formed (food web, for example). The biocenosis along with the biotop creates together the ecological system called ecosystem.

Carcinogens – the chemical, physical and biological agents causing cancer (tumour). Most carcinogens are mutagens, because a first step of the carcinogenesis (process creating a cancer) is the mutation in the gene controlling cell divisions.

Colony – the group of cells visible with the naked eye on the solid medium (e.g. the agar medium) formed by cells originating from the initial unit - which can be one or more cells. If this is from a single cell then the colony is the pure strain.

Electrophoresis – the technique of separation of macromolecules (nucleic acids, proteins) within the electric field created between the anode and the cathode. The molecules with the negative charge migrate to the anode, and molecules with the positive charge - to the cathode. The separation is achieved due to differences in the speed of the migration of ions, which in turn depends on their mass, shape and on the charge.

Free radicals – labile, extremely reactive forms of molecules with unpaired electrons, e.g. peroxyl radical $O_2{\cdot}^-$. Free radicals form eg. during UV radiation. Their high oxidative reactivity can cause serious damage to cellular structures. Antioxidants (e.g. β-carotene, vitamins C, E) neutralize free radicals.

Gram staining – the method of the differentiation of microorganisms (mostly bacteria) by staining them with two dyes. Usually crystal violet and safranin are used. At first the cells are stained with the crystal violet and then are decolorized with alcohol. These bacteria which do not decolorize and stay violet are named gram-positive. Those which decolorize and stain with the second dye (safranin) become pink and are named gram-negative.

Immunology – the science about the resistance of organisms.

Mutagens – chemical or physical agents causing mutations, that are the changes in the DNA structure, passed to the next generations (hereditary). Some pollutants are mutagens, e.g. some aromatic hydrocarbons and their derivatives (benzo-a-pyrene), and also some fungal toxins (aflatoxins).

Nucleocapsid – the complex formed by viral nucleic acid and capsid, the protein coat that encloses the nucleic acid. Some viruses (e.g. influenza viruses) are additionally surrounded by a glycoprotein and lipid-containing membrane called an envelope.

Phagocytosis – the process of taking up small particles (e.g. bacteria) by some cells of the immune system of the organism (e.g. macrophages).

Saprophytic microorganisms – microbes feeding on organic matter from dead organisms (as opposed to parasitic microorganisms, feeding on living organisms and causing diseases). Bacteria and fungi which lead to decay of organic debris participate in the circulation of matter in nature.

Teratogens –chemical or biological factors causing disturbances in embryonic development leading sometimes to serious malformations in embryos. Teratogenic properties can be found in some pesticides, fungal toxins and viruses (e.g. the rubella virus during early pregnancy).

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