It is well known that such elements such as mother rock, plants, and microorganisms play a very important role in the process of soil formation. Hence, this paper concentrates on several issues related to the presence of microorganisms in the soil environment, and interaction between the soil, plant, and organisms that live in it.

According to Palczer et al. [1993], soil microorganisms have been part of the soil environment for almost 4 billion years. Many of them took part in rock weathering and soil formation processes. They have lived mostly on the soil surface and inside it. However, a lot of geological ages passed since they managed to get really deep into the soil. Soil is a living place for the microorganisms and other organisms, especially highly developed plants. They constitute one of the five factors participating in the forming and preserving soil fertility. The remaining four factors are: climate, landscape, mother rock, and time. After the decomposition of solid rocks into finer parts with highly developed surfaces took place, there was release of nutrients accompanying that phenomenon. In the initial stages of soil formation processes, deficiency in the N content and bound C in the mother rock appeared on the surface. Therefore, the surface of the soil forming material was inhabited by cyano-bacteria capable of photosynthesis and binding free N. Further soil formation processes led to the accumulation of live and dead cells. That way the accumulation of soil organic matter began.

In the further stages of soil evolution, microorganisms were partly responsible for the formation of some soil chemical and physical properties. They are responsible for the formation of soil aggregates – the most important factor in the control of microbiological activity and the rate of GMO circulation. Aggregate formation was only possible when fibers and polysaccharide chains were formed from plant roots under the influence of microflora activity. Those fibers and threads joined clay particles to form organic-mineral complexes. Soil structure is created when the physical forces (such as: over-drying, shrinking, swelling, as well as freezing and defrosting, animal movements, and compression) joined soil particles into aggregates.
Microorganisms, as well as soil microfauna and mesofauna, which accompany them, are gathered mainly in the surface layer of the soil. Their ability to spread all over the world is very big. Wind, water, and animals transport microorganisms and carry them for long distances. Microorganisms were isolated from rain drops and snow flakes that had come from great heights. Ocean waters also transport garbage for long distances, even to other continents. It is also true for birds, during their travels they carry microorganisms very far. This way almost all species of microorganisms can be transported from one continent to another. It can be observed while investigating the microflora of Antarctica, where those microorganisms stay alive even in very low temperatures.

Most microorganisms that live in the developed soil are chemoorganotrophes. Their main source of energy is C that gets into the soil through that part of plant waste that gets into the soil during consecutive vegetation seasons. Biological processes shaping soil fertility in the land ecosystems rely mostly on the transformation of organic matter. Soil biological activity is mostly related to microorganisms and enzymes extracted by them, as well as to the rate of the biogeochemical changes carried out by them. They circulate and get transformed from non-organic forms into protoplasm and, after they die, into abiotic parts of the ecosystem.

Research work by numerous authors indicate that from several thousand to several hundred million microorganisms can be found in 1 g of soil (counting colonies of microorganisms grown on agar on plates) [Bolton et al. 1993] and up to several billion of them in the wheat rhizosphere [Kobus et al. 1993]. These results are presented in Tables 1, 2, and 3. Majority of microorganisms are in the surface horizon of the soil. Research by Kaiser and Bolag [1990] proved that microorganisms also appear at great depths, especially in places where there are geological deposits such as gas and petroleum. They were found at the depth of hundreds and thousands of meters. Mainly bacteria and only about 2% of eukaryotic microorganisms are isolated from the great depths. It should be emphasized that almost all the physiological and taxonomic groups were found in those conditions. They had survived a very long period of time in specific, abiotic conditions. It is interesting that the isolated single cells were in most cases capable of forming colonies. They can be compared to the organisms now being isolated from the soil surface [Frederickson et al. 1991].

### TABLE 1. Relative quantities and approximate biomasses of soil microbiota and macro- and microfauna in fertile Mollisol

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Quantity per m²</th>
<th>Quantity per g</th>
<th>Biomass (wet in kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>$10^{13}$-$10^{14}$</td>
<td>$10^8$-$10^9$</td>
<td>300-3000</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>$10^{12}$-$10^{13}$</td>
<td>$10^7$-$10^8$</td>
<td>300-3000</td>
</tr>
<tr>
<td>Fungi</td>
<td>$10^8$-$10^9$</td>
<td>$10^5$-$10^6$</td>
<td>500-5000</td>
</tr>
<tr>
<td>Microalgae</td>
<td>$10^9$-$10^{10}$</td>
<td>$10^3$-$10^4$</td>
<td>10-1500</td>
</tr>
<tr>
<td>Protozoa</td>
<td>$10^9$-$10^{10}$</td>
<td>$10^3$-$10^4$</td>
<td>5-200</td>
</tr>
<tr>
<td>Nematodes</td>
<td>$10^6$-$10^7$</td>
<td>$10^1$-$10^2$</td>
<td>1-100</td>
</tr>
<tr>
<td>Earthworms</td>
<td>30-300</td>
<td>10-1000</td>
<td>1-200</td>
</tr>
<tr>
<td>Other invertebrate</td>
<td>$10^3$-$10^5$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**a**Source: F. Blaine Meeting Jr. [1992]; **b**Mostly arthropodes (crustaceas and insects), ascaris, mollusces
Mineral soils are a habitat for eubacteria, archaebacteria, fungi and yeast, micro-algae, protozoas, nematodes, and other microscopic invertebrate animals. Among the listed live organisms, the most important are plants. Macroscopic animals, such as earthworms, arthropodes, and some vertebrates play an important role in terms of soil biology [Curie and Harper, 1990].

Data included in Table 1 indicates that the biomass of microorganisms in soil constitutes from 1.3% to over 3% of total C. It depends on the research method and on the kind of plant cover. It was found that N included in the biomass of microorganisms constitutes greater percentage than in the plant material, for the ratio of C:N in bacteria is 4:1, whereas in the straw of cereal plants it is not more than 100–200:1. Research carried out by Horwath [1993] on the content of various forms of C and N on different levels of the soil profile in a two-year experiment on a poplar plantation (samples were investigated on 0–25 cm, 25–60 cm, and 60–100 cm) showed that the content of N on various levels balanced between 75 and 51 kg, which accorded with the C of the biomass of bacteria and fungi found on the surface level. Fluctuation of the population of microorganisms appears mainly in lower horizons of the profile. In alluvial soils, a decrease or an increase in the population is related to the change of texture, more microorganisms are in the level of silt than in the level of sand, especially coarse sand. The distribution of microorganisms in the soil profile is also affected by the depth of the water table.

Ecological research on microorganisms appearing in soil itself, as well as in rhizosphere concern both the structure and the functions of the ecosystem [Odum, 1971]. **The structure of the ecosystem includes:**

1. composition of biological groups, including the definition of species, number and their biomass, history of life, and spatial isolation of the population;
2. distribution and number of biotic components, such as nutrients and water;
3. range or degree of existence conditions, such as temperature and light.

**Functions of the ecosystem include:** (1) course of energy in the ecosystem and biogeochemical cycles; (2) biological or ecological regulation. According to Odum [1962], the regulation works two ways: it may concern the regulation of organisms by the environment or the regulation of the environment by organisms.

**Influence of plants on accumulation of nutrients for microorganisms.** The rhizosphere effect, which stimulates the growth of the number and quality of microorganisms by organic compounds released by plants, has been known for a long time [Elliot et al. 1984].

The following organic substances deriving from the roots of plants have been found in the rhizosphere of plants [Rovira 1979]:

**Elicitors:** low-molecular compounds (sugars, aminoacids) leaking from untouched cells;

**Secretions:** compounds, which are actively secreted from root cells.

**Plant gels:** derive from different parts of the root, including:

- a) secretions from the Golgi apparatus and from caps of the cells of the growing point,
- b) hydrolizates of primary cells located between the growing point and the epidermis,
- c) elicitors of epidermal cells and root hairs from primary walls,
- d) compounds deriving from microbiological degradation and modification of dead epidermal cells;
4. **Mucogels**: gel material on the surface of roots, built of the mukogel of bacterial cells, metabolism products, organic colloids, and mineral material;

5. **Lysates**: material released by lysis of older epidermal cells.

Particles, which go through the membrane of a cell wall are also secreted through growing points. Gels are highly hydrated and therefore they show fibroidal structure containing carboxyl groups, with which they bind with clayses minerals. Slime is a dominating material produced by roots.

Quantity analysis of $^{14}$C assimilated by plant and their secretions shows that the plants of wheat secrete 2 to 16% of fixed CO$_2$ into soil. That CO$_2$ is then accumulated in cells of microorganisms and in their biomass.

According to Barber and Martin [1976], roots of barley secreted 5 to 10% of photosynthetic compounds in sterile conditions, but at the presence of microorganisms, the amount of those secretions rose to 12 to 18%. It means that microorganisms inhabiting roots increase their permeability. Increased permeability of roots might be the result of root damage or increased production of hormones affecting the change of plant physiology.

**Influence of roots on physical properties of soil.** Investigating the effect of plant roots on soil, it can be stated that usually in an early stage of development they grow in less compacted spaces, like pores or old dead root holes, although in further development, the plants may even penetrate mother rocks [Foster, 1986]. Mineral situated near roots are subject to intensive weathering. Amorphic oxides of iron and aluminum gather around roots [Sarkar et al. 1979]. Nutrients, together with water go through the pores in the rhizosphere into plant roots. Usually, there is less water and nutrients in the rhizosphere than in soil without roots.

Plant roots also influence the level of water in the rhizosphere available for mesophytic plants and it should equal 2.00 MPa, while in the rhizosphere of xerophytic plants it should be about 4 MPa [Foster and Bowen, 1982]. Therefore, the water in the rhizosphere might be the factor, which controls the composition and the activity of microorganisms of the rhizosphere. Water potential of the rhizosphere depends on the intensity of transpiration and its low level can last for a shorter or a longer period of time, during which the microorganisms of the rhizosphere should be able to survive.

**Influence of roots on chemical properties of soil solutions and microorganisms.** Plants not only secrete organic substances, but also non-organic compounds which affect the environment and, therefore, affect also the microflora of the rhizosphere. Plant roots can take selectively ions and, therefore, they change the pH and Eh of soil solutions, as well as the concentration of nutrients. Content of soluble compounds of C also changes, which, in turns, affects the activity of microorganisms and the source of energy. In such conditions, solubility of complexed cations may rise. Concentrations of Mn, Zn, and Cu in the solution has significantly changed in the field, where barley had been cultivated during the vegetation period [Linehan et al. 1985].

In rhizosphere, there are great changes of the pH comparing to soil without roots. This change is a result of releasing H$^+$ or HCO$_3^-$ ions by roots during the uptake of nutrients, secretion of CO$_2$ during respiration, and releasing organic acids and aminoacids by roots [Marschner, 1986]. It has been proved that the differences of the reaction in the rhizosphere of wheat and soil free from roots had been able to reach 2.2 units if fertilized with NH$_4$ instead of nitrate fertilizers. The decrease in the pH can also be profitable in terms of plant protection against
Gaeumannomyces graminis. That pathogen is very sensitive to acidification of the environment [Smeiley and Cook, 1973]. A pH sensitive organism is also Azoto-
bacter, which does not appear in direct connection with roots of cereal plants [Maliszewska, 1961; Kobus et al. 1981].

Respiration of roots and organisms in the rhizosphere of plants creates an environment containing less O$_2$ and having weaker oxidation-reduction potential than in soil without roots. It is supposed that this is the reason why anaerobic microhabitats are created in soil near roots. As it was proved, that was also the reason there was an intensive denitrification in the rhizosphere of plants, even when soil moisture did not reach full water saturation [Smith and Tiedje, 1979]. Accelerated denitrification is caused by the presence of soluble organic substances in the rhizosphere which increase the rate of respiration and, in turns, cause the decrease of the supply of O$_2$ and fast exhaust of nitrates, which are electron acceptors. This indicates that optional denitrificators are able to adapt to the rhizosphere better and to survive even much greater fluctuation of O$_2$ than it occurs in the rhizosphere. It should be stressed that microorganisms use about 70% of the O$_2$ supply in soil and plant roots use only 30%.

**MICROORGANISMS IN RHIZOSPHERE AND THEIR EFFECT ON PLANT GROWTH**

The greatest concentration of microorganisms is in the rhizosphere of plants and they appear mainly in the area directly surrounding the roots. Rhizosphere is divided into ectorhizosphere – the outer sphere, and endorhizosphere – the internal sphere where the invasion and the colonization of cork tissue cells and epidermis cells by microorganisms takes place [Balandreau and Knowles, 1978]. In places, where mycorhiza fungi are, that part of the rhizosphere is named mycorrhizosphere [Linderman, 1988] and in places, where nodules develop under the influence of Frankia, it is called actynorhizosphere or actynorhiza [Torrey and Tjepkema, 1979]. In the rhizosphere of papilionaceous plants there also are Rhizobium, which create nodules. On the surface of the root, together with a thin layer of soil contacting directly the roots, it is called Rhizoplana [Clark, 1949]. It is practically very difficult to notice the difference between this layer and the rhizosphere.

The effect of rhizosphere not only concerns the acceleration of microorganism growth and the increase in the density of population of rhizosphere, but it also concerns the growth of kind and species variation comparing with soil without roots [Foster, 1986]. The rhizosphere effect is the ratio of the population of rhizosphere to the population of R/S soil. Greater quantity of microorganisms in rhizosphere depends on the micro-environment conditions and the range of the influence zone may even exceed 2 mm from the surface of the root [Foster and Bowen, 1982]. According to Richards [1976], R/S for bacteria, fungi, protozoa, and algae was correspondingly 10-50, 5-10, 1-3, and 1-2.

**Occurrence of microorganisms**

Although the growth of microorganisms is stimulated in rhizosphere and rhizoplana, rhizoplana is covered with the layer of microorganisms. As research
of many authors [Rovira, 1979 and others] indicates, only 4 to 10% of the surface of roots is inhabited. Foster [1986] investigated the general quantity of bacteria and their morphological types occurring in the rhizosphere of clover (Trifolium subterraneum L.). Morphological types of those microorganisms were defined using a transmission-electron microscope in ultra-thin stripes. The results are shown in Table 2.

Data presented in Table 3 showed that microbes of the rhizosphere are gathered mainly in the soil adhering to the roots and it is only at the stage of full maturity, when little higher number of them was stated on the plant root surface and in the zone of epidermis and cork cells.

The research on the appearance of Azotobacter in the rhizosphere of winter wheat proved its numerous presence in the soil adhering to the roots. With further washing out and while investigating further fractions, no cells of Azotobacter were stated (Table 3). It should be mentioned that the soil outside the roots had the pH of 7.2 and the effect of rhizosphere causing a decrease in the pH, was not able to change the soil reaction in the way that in would make it impossible for the bacteria of the Azobacter kind to develop [Kobus et al. 1999].

Colonization of plant roots by microorganisms

Dominating microorganisms in rhizosphere, according to Alexander (1975), were short, Gram negative bacteria including: Pseudomonas, Fulvobacterium, and Alcaligenes. Our research indicates that in the rhizosphere of winter wheat, there are Gram negative strains of bacteria: Pseudomonas, Alcaligenes, Erwinia, Jan-

---

**TABLE 2.** Morphological types of microorganisms in relation to the distance from the root surface in μm based on the ultrastructural morphology and total quantity at various distances from the roots of Subterranean Clover (Trifolium subterraneum L.) as determined by the transmission of electron microscopy of the ultra-thin sections

<table>
<thead>
<tr>
<th>Distance in μm</th>
<th>Morphologically distinguished microorganisms</th>
<th>Quantity (× 10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>8</td>
<td>120</td>
</tr>
<tr>
<td>0–5</td>
<td>11</td>
<td>96</td>
</tr>
<tr>
<td>5–10</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>10–15</td>
<td>3</td>
<td>34</td>
</tr>
<tr>
<td>15–20</td>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>

aSource: Foster, 1986

**TABLE 3.** Total number of bacteria and fungi present in the different fractions of the rhizospheres of winter wheat a (in percent)

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-nd leaf</td>
<td>4th leaf</td>
</tr>
<tr>
<td>1</td>
<td>95.9*</td>
<td>94.8</td>
</tr>
<tr>
<td>2</td>
<td>1.9</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>4</td>
<td>0.007</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*Source: Department of Soil Microbiology of the Institute of Soil Science and Plant Cultivation.

aSoil adhering to the roots, bacteria or fungi on the root surface, c number of bacteria or fungi in the roots after homogenisation, d relative number of bacteria or fungi in the roots after surface sterilisation and homogenisation.
TABLE 4. Numbers of *Azotobacter* cells in the winter wheat rhizosphere (per 1 g of the soil)

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Wheat growth stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-nd leaf</td>
</tr>
<tr>
<td>1</td>
<td>6690</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

See explanations for Table 3.

thinobacterium, Xantomonas, Klebsiella, Flexibacter, and Acinetobacter, as well as Gram positive strains: Bacillus, Micrococcus, and Gram changeable bacteria of Coryneform and Actinomycetes group.

From Table 4 it results that in the rhizosphere also fungi occurred. They predominantly gathered in soil adhering to the roots. The following genera were most often isolated: Acremonium, Alternaria, Arthinum, Fusarium, Gliocladium, Mucor, Penicillium, Phoma, Trichoderma, Verticillium, as well as yeast and non-sporous fungi. From the rhizosphere of plants growing actively there were isolated Penicillium, Trichoderma and Verticillium, while from the rhizosphere of maturing winter wheat were strains from genera of Mucor, Gliocladium and Penicillium.

Mechanism of colonization

Colonization of roots in soil often starts with plant roots infection by microbiota inhabiting the surface of soil organic particles or introduced in a vaccine on seeds or roots [Bowen, 1980]. Mechanism of colonization of plant roots by microorganisms is not well known. That process is affected by many factors, including the microorganisms’ ability to adhere to root cells. Polysaccharides occurring on the surface of bacterial cells are also of great importance especially for bacteria living in association with the plant. Similar mechanism of root colonization is used by such bacteria as: Agrobacterium tumefaciens, causing nodulation of many plant roots, as well as by bacteria producing nodules by *Rhizobium* sp. and *Frankia* [Smith et al. 1987; Thomashow et al. 1990]. Same thing with the strain of *Pseudomonas putida* - which very intensively colonizes the roots. *Phaseolus vulgaris* was agglutinated by glycoproteins of bean roots [Anderson et al. 1988]. The way of colonization by agglutination was proved by comparing the mutants of *Pseudomonas putida* devoid of the ability to agglutinate by bean roots, which has clearly caused the smaller colonization of roots than by wild strains. Other mechanism of bacteria’s contact with roots is bacteria sticking to the root surface using fimbriae built of proteins, growing out of the cells, for example: Klebsiella and Enterobacter sp. Similar mechanism was stated in *Bradyrhizobium japonicum* [Vesper et al. 1987]. These authors stated that the transpozone mutant of *B. japonicum*, which created piles, colonized 2.5 times more soybean roots than the wild strain.

Plant roots have a direct influence on the microorganisms inhabiting soil or introduced into it. It was stated that the critical point of localization for microorganisms is the surface of plant roots - which is rhizoplane. At present, it is known
that the outer surface of the endodermis, also called the epidermal tissue and the cork tissue of the roots, as well as the cap and the growing point are the biotic habitat for microorganisms, but only for those other than symbiotic and pathogens [Bolton et al. 1993].

Various studies indicate that microorganisms living on the surface of roots inhabit caves, which occur on the roots in places of tissue joints. The saprophytic bacteria rarely appear in the epidermal tissue and the cork tissue of young plants. They can be found only in greater numbers as the roots become older (Table 3). The dying roots create pores or air tunnels which are colonized by fungi. The rhizosphere is colonized by many different forms of microorganisms, although there are more G- bacteria *Pseudomonas, Achromobacter*, including the denitrifiers, than G+ and G changeable (*Arthrobacter*). Intensive development of microorganisms in the rhizosphere causes the development of micro-biota, especially the protozoa feeding on bacteria or roots.

**Substance provided by roots**

Analysis of organic materials proved that in the neighbourhood of roots there was a great supply of aliphatic compounds such as amino-acids; organic aliphatic and aromatic acids; and phenol derivatives. In addition, there are soluble substances in the rhizosphere which transform into non-soluble compounds in roots (this concerns cellulose, lignin, and proteins). Some plant species produce attractants and repellents. For instance, asparagus produces diffusive glycosides which are toxic to nematodes. Some plants produce diffusive compounds of different stages of biostability or biocides dangerous to saprophytes or pathogens of roots. Among phytoalexins there are: tomatin, alicin, pisatin, phaseolin – produced by potatoes, onion, pea and bean correspondingly [Lynch, 1990].

**Survival rate of bacterial strains introduced in the vaccine**

More and more often introduced into soil are strains of microorganisms which are very important in processes of organic matter transformation, detoxication of the environment or plant protection. Research on the survival rate of Rifomycin (Rif") and Streptomycin (Str") resistant strains: *Pseudomonas fluorescens* No. 107 and 21dc and *Bacillus* B192 and B184 in fresh soil in the conditions of the growth chamber proved that anti-biotic mutants (in a 4-week experiment) showed decreasing titre of these bacteria in the soil. For example, the *Psedomonas fluorescens* strain No. 107 had lost about 50% of its size during 14 days of incubation, whereas the size of the strain of *Pseudomonas* No. 21dc had decreased by only 25%. In those condition, very stable were the strains of the *Bacillus* sp. genera No. 192 and B194. During the period of 28 days, no decrease of the population of *Bacillus* No. 192 was stated and there was a minimum decrease in the size of *Bacillus* No. B184 (Figure 1). That research indicates that *Pseudomonas* are more sensitive to the antagonist activity and to the metabolites of the native microflora than the *Bacillus* genera, although there is a significant strain difference even within the species [Kobus 1999].

Literature data indicate that only a small percentage of organisms introduced in a vaccine can survive a longer period of time in soil. Even though small number
Interaction between soil, plant, and microorganisms

FIGURE 1. Survival of bacteria introduced into the soil in growth chamber studies with wheat

of microorganisms introduced into the soil environment is able to reactivate when stress conditions of the environment change.

At present many investigations are carried out on the possibility of introducing active microorganisms, obtained through genetic engineering, into soil, in order to reach certain effect in soil. It has been known for a long time that soil creates conditions appropriate for exchanging genetic material. The possibilities of genetic exchange between the introduced and the native organisms must be significant since many countries have made the decisions on limiting the usage of those strains in field conditions. It is also the reason why many investigations are carried out on the ecological confrontation of native microorganisms and the ones changed genetically in laboratory conditions.

In order to avoid introducing strange bacterial genes or the ones used for industrial purposes into the soil environment, microorganisms containing lethal genes in GEM have been constructed which destroy bacterial cells after having performed a specific duty. In order to do that the fusion of lethal genes was introduced into the system of enzymatic regulation and specific substrates through
the promoter of gene degradation. In this case, the production of enzymes in the environment and the decrease of the lethal function of the gene will last for as long as the substrate (for example the degraded herbicide) is controlled by the special enzymatic system. At the lack of the enzyme induction, the expression of the DNA shows up in the killer gene which causes great dying rate of the bacterial cells. Hunger may also cause the expression of the killer genes and the death of the bacteria.

If we consider introducing microorganisms, which contain the biological system including suicidal genes, into the environment the following factors are to be considered before that introduction:

1. the introduced genes may not disturb other engineering microorganisms;
2. the system should be usable for many groups of bacteria;
3. killing effectiveness must be high with small chances of becoming resistant.

Two-system lethal genes were received. The first one includes the family gene; that family produces proteins consisting of the average of 50 amino-acids which interfere with the membrane potential. The activity of those genes causes a decrease of the membrane potential and causes the death of the cell. That effect concerns only some of the strains. The second system concerns the incorporation of the gene responsible for nuclease production. That gene causes partition of the genetic material. The example is outer-cellular nuclease *Serratia marcescens*. The cutting structure originates from nuclease gene and fusion of expression vector through transposon which infact leads to production of nuclease. The constructions listed above lead to an inhibition of growth of the strain cells and the genetically changed microorganism is incapable of surviving at the lack of an appropriate substrate. Introducing the organisms containing the lethal gene does not guarantee that a 100% of those bacteria die, but it is sure that their number falls down to the lower level than needed for nature infection [Paul & Clark, 1996].

**Influence of the rhizosphere microflora on plants**

We can often meet a statement that plants are able to live without microorganisms. It is also well known that they may live in sterile hydroponics under the condition that we provide them with a sufficient amount of nutrients in an available form with appropriate concentration, and a proper amount of water and access to light. Unfortunately, it is difficult to provide such conditions, both in vitro and in conditions of poor soils. Therefore, plant development depends on the fertility of soils, including formation and preservation of that fertility, in which very important is the role of microorganisms. They are regulators of biochemical changes of the organic matter through bacteria which affect the preservation of the stable level of humus in soil, they regulate water and air relations in soil. Microbes ensure an even inflow of nutrients during the entire period of plant vegetation through assimilation and decomposition of nitrogen, phosphorus, and other compounds. A very important function is therefore performed by microorganisms in terms of creating an appropriate physical and chemical environment for particular plants [Balicka et al. 1964].
Function of the microorganisms

Biological processes forming the fertility of soil in terrestrial ecosystems rely mainly on the transformation of the organic matter. They are mostly related to the microbes secreting enzymes and to the speed of carried out by them biogeochemical changes in the circulation of elements which transform from inorganic or organic inanimate forms into protoplasmic bindings. After their death they return to the abiotic part of the ecosystem. Microbes carry out various geochemical processes. The course of these processes and their effect on the environment depend on their activity. They play the main role in terrestrial ecosystems covered by forest, arable land, grassland, and even in ecosystems of deserts and tundra [Kobus, 1995]. Each cell and each species, one way or another, changes the chemical composition of their own ecological niche. These changes are clearly visible when enzymes and the developed metabolites are so strong that the changes induced by them become visible [Smith et al. 1992; Doran et al. 1994].

According to Alexander [1985], known are several categories of microbiological activity of geochemical significance. Most important in terms of ecosystems functioning are:

a) **mineralisation of organic compounds** – processes which lead to the transformation of the complex organic matter into a simple mineral form. These are one of the most important processes which condition the development and the functioning of the terrestrial ecosystems. Biochemical complexity of the ecosystem decreases in the mineralizing process. Mineralizing is often related to as regeneration of nutrients.

b) **immobilisation (fixation)** consists in binding of inorganic forms of an element into a complex organic bonds as the result of the element assimilation and including it in the organic form by microorganism cells. They are processes opposite to mineralisation and both groups of these processes condition the direction and the intensity of biogeochemical cycles.

c) **oxidation** – microbiological processes connected with organic changes. The example could be the oxidation of the organic matter by heterotrophes. This process is accompanied by the release of nitrogen, sulphur, iron and hydrogen and oxidation of the compounds mentioned above by chemoautotrophic bacteria. There are also processes of oxidation in which microorganisms do not receive the energy necessary to grow or they receive it only in small amounts. The example can be oxidation of hydrogen sulphide by photoautotrophes or heterotrophic microbes which oxidize sulphur or ammonia.

d) **reduction processes can join energetic changes in cells**. In this process, microorganisms use substances which are included in protoplasm. Some reduction processes happen as the result of environment modifications caused by microflora developing in it, for example as the result of oxygen exhaustion, the $E_r$ of the environment decreases.

e) **transformation of organic matter in which chemical compounds in gaseous form are emitted during oxidation**. Those processes are accompanied by the change in the concentration of different elements in the ecosystem. Evolution of gaseous forms is accompanied by losses of carbon, decrease in nitrogen reserves due to denitrification, evolution of hydrogen sulphide, ammonia, and methyl forms of some heavy metals (Table 5).
TABLE 5. The elements content in compounds metabolised by microorganisms

<table>
<thead>
<tr>
<th>Processes</th>
<th>Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineralisation</td>
<td>S, N, P, S, K, Si, Fe, and others</td>
</tr>
<tr>
<td>Immobilization</td>
<td>C, N, P, S, K, Si, Fe, and others</td>
</tr>
<tr>
<td>Oxidation</td>
<td>C, N, P, S, H, Fe, Mn, Cl</td>
</tr>
<tr>
<td>Formed dissolved forms</td>
<td>P, S, Fe, K, Ca, Mn, Mg, Al, Cu, Zn, Co</td>
</tr>
<tr>
<td>Precipitation</td>
<td></td>
</tr>
<tr>
<td>Fixation</td>
<td>S, Fe, Ca, Mg, Mn</td>
</tr>
<tr>
<td>Formation of gaseous forms</td>
<td>C, N, H, O, Se, As, Hg, Pb*</td>
</tr>
</tbody>
</table>

* The formation of (CH₃)₃Pb is not to be evidenced.

f) **formation of geological strata.** Due to the processes of oxidation and reduction, microorganisms have great influence on the creation of geological formations. Some of those processes may occur as result of physical-chemical transformations. However, at least one product of the reaction originates from microbiological activity or sediments of biomass residues. Evidence has been found that microorganisms had taken part in the formation of sulphur, crude oil, and coal deposits.

   g) **production of organic substances capable of forming chelate bindings or bindings polymerizing metals.**

   h) **adsorption of inorganic substances** – for example oxides or hydroxides of metals.

   i) **fractioning of isotopes** – thanks to selective uptake of one isotope by microorganisms from the mixture of microbes occurring in nature. In microbiological products, those isotopes are gathered in different proportions.

Origin and components of soil organic matter

The scheme illustrating the role of living microorganisms in the production of soil organic matter is shown in Figure 2.

One of the most important processes carried out by microorganisms is formation of soil organic matter. Figure 2 presents the role of microbes in the production of soil organic matter. During the decomposition of plant and animal residues some by-products are formed. These products undergo processes of humification and a very specific form of organic material is created which is called humus. The amount of humus in soil decides about its fertility and biological activity of soil. The amount of humus depends on the conditions of organic residue decomposition in the soil environment.

Traditionally, soil organic matter is separated from native clay colloids. The amount of organic matter in the soil of the moderate zone, northern regions, and tropical forests of the lowlands are correspondingly 202, 208, and 287 t × ha⁻¹. Relatively little of this matter was found in tropical grass systems, desert forest areas, and arable land (from 42 to 79 t × ha⁻¹, respectively). Decomposition of organic matter is reduced by the cold climate in soils of tundra and in bog areas, where the cycling of C lasts 490 and 520 years. In other ecosystems it ranges from 10 to 91 years. The fastest decomposition takes place in tropical grass systems in which the amount of organic matter is the smallest.
Bacteria as a biological factor controlling growth and plant protection

During over 100 years of research on soil microbes, it has been shown that soil microbes favour the development of plants and their health. Some of them are used for a relatively long period of time in industry and agricultural practice.

Using microorganisms by humans has been dated since prehistoric times. They were used for milk fermentation, fruit juices, bread baking, food conservation, and other purposes. During the last decade of the 19th century and at the beginning of the 20th century vaccination of soil with microbes binding free nitrogen by free bacteria, such as *Azotobacter chroococcum* were initiated. Many species of those bacteria were isolated and described. In the last decade, they were divided into two genera: *Azotobacter* and *Azomonas*. They bind N\textsubscript{2} in the amount of about 1\% in relation to the used glucose.

In tropical soil of India, Japan, and Africa, even in soils of southern France and Yugoslavia, beside *Azobacter* also a tropical homologue of the *Azobacter*, called *Beijerinckia* can be found. In favourable conditions they bind nitrogen in the amount of 0.5 to 2\% N\textsubscript{2} in relation to the used sugar [Dommergeues et al. 1984].

*Derxia* – is also a bacteria which binds atmospheric nitrogen in tropical soils. Its efficiency is very high, for it binds 4\% of N\textsubscript{2} in relation to the used glucose [Roy and Subir Sen 1962].

Beside the oxygen bacteria also anaerobes occur in soil and water. Most common in soils are: *Clostridium butyricum*, *C. pasteurianum*, *C. beijerinckia*, and other species. The included list does not comprise all known species.
In soil and even in deep gas and oil drillings, there are also anaerobes which reduce sulphur and bind N\textsubscript{2}. They are *Desulfovibrio vulgaris* or *Desulfobrium* sp.

A bacterium which binds free nitrogen and lives in an association is *Azospirillum*. Becking [1963] stated that *Azospirillum* is able to bind atmospheric nitrogen. It appears on plant roots and is able to penetrate the cork layer of the roots [Nur et al. 1980; Watenabe and Liu 1983; Christiansen-Weniger and Van Veen 1991; Król and Kobus 1997]. The used methods of labeled nitrogen solution (isotope \textsuperscript{15}N) proved that plants could receive N in the form of NH\textsubscript{4}\textsuperscript{+} during its binding [Okon et al. 1983; Christiansen-Weniger and Van Veen 1991]. At present there are attempts to induce the roots of wheat and corn, on which there is *Azospirillum* of p-nodules or tumors, on seedlings of plants treated with artificial auxine. Bacteria which bind free nitrogen and which bind several times more N\textsubscript{2} than *Azospirillum* living on the surface of roots gather in p-nodules [Christiansen-Weniger et al. 1991; Król and Kobus 1997].

On the surface of soil and alkaline rocks on the whole Earth there are photosynthetic bacteria binding free N\textsubscript{2} called *Cyanobacteria*. They were previously classified into algae. It appeared that during binding of molecular nitrogen they transmit directly into the environment the nitrogen in the form of ammonia, amino-acids, or fine peptides. Secretion of those substances is of great practical importance for plants and microorganisms living within the same environment.

**Binding of free nitrogen by bacteria living in symbiosis**

Binding of free nitrogen by symbiotic bacteria is especially important for the terrestrial ecosystem. Many plants gain much profit from bacteria binding free nitrogen and living in association with them. Symbiosis is established by *Rhizobium* with *Papilionaceae*, *Anabaena* – with water fern *Azolla*, *Frankia* with alder trees and other trees and shrubs. The most commonly known is the symbiosis of *Rhizobium* with bacteria forming nodules on roots of the *Papilionaceae*.

The *Rhizobium* family consists of four clear genera: *Rhizobium*; *Azorhizobium*; *Sinrhizobium* – fast growing strains; and *Bradyrhizobium* – slow growing strains. Specific is receiving proper signals which are exchanged between *Rhizobium* and the host plant. Roots of papilionaceous plants secrete a mixture of metabolites (mainly flavonoids [Peters et al. 1980; Redmont et al. 1986]), which are received by bacteria and cause the activation of *Rhizobium*. This leads to the induction of a complex of changes in plant roots. Incubation of proper plants with compatible strain of *Rhizobium*, of which respective genes fix synthesize morphological and biochemical status, lead to binding of N\textsubscript{2} [Fisher 1994]. In plants, the specific *nod* genes are induced in various stages of interaction and the products of those genes – nodulines – probably play an important role in creating nodules and in preserving them [Wijn et al. 1993]. In optimum conditions, papilionaceous plants can bind from several dozen to several hundred kg of N/ha. For example, lucerne II3 – up to 297 kg of N\textsubscript{2}/ha, red clover – from 75 to 171 kg, soybean – from 57 to 105 kg/ha.

It was calculated that actinorhizous plants could bind from 60 to 320 kg/ha of N\textsubscript{2} per year [Newton et al. 1986; Trappe 1987].

In the south-eastern region of China and in northern Vietnam, the symbiotic system of *Anabaena-Azolla* is a basic green fertilizer used for rise cultivation and for duck, fish, and pork breeding. The system mentioned above gives the possibi-
lity to provide from 30 to 40 kg of N₂/ha per vegetation season. Therefore, it is an additional amount of nitrogen to the basic dose of that component.

In the 1950’s, a phosphobacterine containing a bacterial strain of *Bacillus megatherium* var. *phosphaticum*, as well as vaccines of bacteria decomposing aluminum silicates by bacteria *Bacillus megatherium* var. *silicum*, was widely produced and used.

At the end of the 19th century mycorrhiza was discovered – it is a symbiosis between plant roots and fungi. Presently, it is a huge field of knowledge. There are many kinds of mycorrhizae, but the most common are endo- and ectotrophic ones. Mycorrhiza VAM endotrophic initiates fungi penetration into plant roots and formation of abruscules which enables substrate exchange (predominantly phosphorus and carbohydrates) between partners. It is mycorrhiza which concerns about 85 to 90% of terrestrial plants.

Especially interesting is the issue of phosphorus uptake by mycorrhiza plants. Phosphorus is necessary for plants in relatively big amounts, and because it is strongly adsorbed by soil particles - there is relatively little phosphorus available for plants in soil. The outside mycelium wraps closely the soil particles which allows to release greater amounts of phosphate ions from soil. Due to slow diffusion they reach plant root [Johnson et al. 1991; Jakobsen et al. 1994]. In mycorrhizae plants, a positive answer is faster growth of plants in soils with a relatively low concentration of nutrients, such as P. The lowest concentration of P in the soil solution, at which various field cultivated plants could still live in a symbiosis with mycorrhiza fungi, ranges from 0.1 µg/ml to 1.6 µg/ml for soybean, cassava, and *Stylosanthes*. Therefore, the plants mentioned above are obligatory mycotrophes.

According to Sylvia [1992], the length of outer hyphes penetrating soil ranges between 1 and 50 m per 1 g of soil, and most often it is between 5 and 15 m. The length of the mycelium is about 100 times greater than the length of the roots in similar volume of soil. Growth of non-root shreds is, in the great part, stimulated by the soil organic matter – it might be that the stimulation results from the increased activity of microorganisms [Wyss and Bonfante 1993].

Presently, it is supposed that almost all gymnosperous plants and about 85% of angiosperous plants may create mycorrhiza which allows or helps to survive and to reproduce. Green plants mostly create endomycorrhiza of the vesicular-arbuscular type in symbiosis with fungi of the *Zygomycetes* class - order *Glomales* and *Gigasporaceae*. They are obligatory biotrophes which uptake nutrients from the living cells of the host. Interaction between the partners of the symbiosis, a plant and a fungus, leads to severe changes both in the underground and the above-ground parts of plants. They are expressed by changes in quantity and quality of plant root exudates and by supplying plants with nutrients, such as phosphorus, nitrogen, and microelements. In mycorrhizeous plant also increases the resistance to some pathogens or stresses (like water deficit stress, heavy metal pollution). To obtain the symbiosis between the plant host and the fungus VAM, molecular signals must appear. The points of receiving those signals can be spores or intra-root receptors. They induce genes in the plant, which code appropriate physiological and anatomical changes, necessary for the effective symbiosis to exist.
At present, more and more preparations of microorganisms appear on the market which are used in nutrition industry, added to soil as factors increasing plant nutrition, or are used in plant and environmental protection.

Great role is played by the fast developing genetic engineering which offers greater and greater possibilities. It is the first time there are big possibilities of using bacteria in a practical scale. Alarming is the fact that there may be a genetic transfer of genes from organisms created in laboratories to native microorganisms inhabiting soil. Since plants can be controlled much easier than bacteria, genes deriving from microorganisms or other sources are delivered to plants.

**BIOCONTROL**

Biocontrol consists in using one organism against another in order to ensure the development of one of them, like in case of parasites or weeding in agricultural cultivation. Biocontrol may be defined as a helping process or a stimulator appearing naturally in the biological war. For many years that function was performed by chemicals being the basic means of controlling weeds and illnesses of cultivated plants. It should be mentioned here that the use of chemicals is connected with great risk of them getting into the food chain. It is also connected with great costs of purchasing chemical pesticides and increasing restrictions towards their use. Therefore, presently the research on biological control are being intensified.

Mechanisms of biological control are not yet fully recognized; verified is their specific effect on pathogens. Usually, there are three mechanisms used in biological control:

1. dumping of the target organism by antibiotics, enzymes, or biocides (antagonism);
2. dumping by changing nutrients towards its non-availability for pathogens (competition);
3. dumping by parasites or reptiles — pests and parasites (exploitation).

Additionally, microorganisms can interact with plants and increase their resistance to parasites, pests, or weeds — making them more sensitive to damage or death. Bacteria included in such interactions are considered organisms promoting plant growth and are called „rhizobacteria”.

An example of the mechanism of antagonistic influence can be production of anti-biotics by *Pseudomonas fluorescens* — this organism produces phenazine antibiotics which control the wheat stake-all. Known is also the antagonist influence of one group of fungi on another through production of antibiotics antagonistically influencing the other fungi. The example here could be *Chaetomium globosum* which produces cochlinidol — the inhibitor of the development of *Fusarium* causing rust in corn seedlings. *Myrothecium verrucaria* and *Clionicodium virens* produce vericarine and glycovirine. These two metabolites dampen the development of *Rhizoctonia solani* and *Pythium ultimum* — they are fungi causing wilting of potato and tomato plants. *Bacillus thuringensis (Bt)* produces metabolites of wide spectrum of influence on larvae of green plants insects. A toxic metabolite is crystal protein which, during digestion by insects at high pH, causes damage to cell membranes of the digestive system. The breeding mass contains spores of the *Bt* toxin, with which it is soluted before spraying ornamental
plants and trees against specific insects. *Pseudomonas fluorescens* is a fungicide microorganism the cultures of which are used for vaccinating seeds of roots. Maize culms are protected by using mutual endophyte of maize *Clavibacter xili*.

**Competition**

It is the third mechanism where two microorganisms requiring the same source of the easily available nutrient are involved. Great success for the microorganism is to weaken the possibilities of development of another. All microorganisms develop in stress of the competition using the principle of saving their own existence and in this case it becomes a natural biocontrol. Lack of competition gives the „boomerang” effect. For example — garden soil, partly sterilized with the fumigation method, eliminates pathogens. However, as the plants are cultivated we can observe an increased loss of plants on sterile ground instead of decreasing losses. It is a result of a lack of competitive microorganisms in the environment in relation to the in-going pathogen which can develop in these conditions with no problems. An example of competition is also competition for Fe.

In some soils, there is a shortage of available Fe. Inoculation of soil with bacteria of the following genera: *Pseudomonas, Erwinia, Nocardia, Penicillium,* and *Trichoderma* [Księżniak and Kobus, 1993] allow to control the development of *Fusarium oxysporum* and *Pythium ultimum*. The theory says that siderophores create chelate bindings with Fe which decreases its availability to phytopathogens. There is much data concerning competition between fungi for places of plant infection. It was stated that the avirulent strain of *Fusarium oxysporum* had clearly controlled the infection of plants by virulent strains of *F. oxysporum*. Up-to-date there have been little preparations on the market which had a developed mechanism of competitive organism control. The same thing concerns *Agrobacterium tumefaciens* which causes nodules on roots of various plants. They may not have a possibility to infect, if there is *Agrobacterium radiobacter* in the plant. It is a K85 strain and it blocks the place of root damage from the *Agrobacterium tumefaciens* pathogenic strain.

It has been stated in recent years that on the way of interaction between plants and symbiotic microorganisms and pathogens, economically important changes in plants may occur. One of the systems can be the activity of epiphytic microorganisms which live on the surface of leaves or in their stomata. This way bacterial genes can be delivered to the plant through „ice-nucleation activity”. These organisms could have influence on yielding of cultivable plants. Changes induced by this phenomenon are not characterized in detail and therefore they are not discussed in review articles [Baron and Zambryski, 1995]. The second possibility of interaction between plants and microorganisms is symbiosis – in this system both partners have profits. In the third case, the pathogenic microorganism can use up the resources of the host and this way results in serious damage of the host leading to its death.

It was stated that microbes recognize proper plants through signal molecules given by plants. For instance, *Agrobacterium* recognizes the secreted molecules through wounded plant roots. Signal molecules, in case of a damaged root, are: aceto-syringe alcohol and α-hydroxy-aceto-syringe alcohol. Microorganisms can
recognize these signals and in response they induce virulence system (vir) in their cells or specific genes. Well known is the path of transformation of many plants under the influence of *Agrobacterium*. The very important factors for the development of these processes are the presence of sugars and the low reaction of the environment.

These molecules are supposed to be secreted by damaged cells as secondary metabolites of degradation or reparation of lignin. The discovery of those molecules is an example of signal exchange between plants and bacteria, and this deepens our theory on interaction between various organisms. Well known is the path of transformation of many plants under the influence of *Agrobacterium*. This organism is a natural factor of genetic engineering. *Agrobacterium* is a microorganism which is attracted by secretions from damaged roots during digging out fruit tree seedlings from soil, for instance. Signal compounds induce gene *Vir A* which, in turn, induces gene *Vir G* and many others which penetrate through nucleus membrane and integrates itself with plant genome. Bacterial genes introduced into the plant genome code the synthesis of phytohormones which cause an increase of nodules on the roots of the infected plant. In addition, plant roots synthesize compounds of organic acids with amino-acids or sugars. Those compounds are immediately metabolized by *Agrobacterium*.

The ability of *Agrobacterium* to perform the processes mentioned above is used by plant growers, who use non-pathogenic strains to transform dicotyledonous plants as a routine.

It would probably be a great achievement to brake the barrier of resistance of monocotyledonous plants to transformation under the influence of *Agrobacterium*.

Also, chemical signals between *Rhizobium* and their host plants were stated. Most of *Rhizobium* have a small group of host plants which create nodules. They are specific sub-groups of papilionaceous plants. This specific character is based on the possibility of recognizing the signals. Roots of papilionaceous secrete a mixture of secondary metabolites, most of which are flavonoids [Peterson et al. 1986; Redmont 1986], but some of them are betaines [Philip et al. 1992]. These signals are understood by the family NodD with transcriptional activators of *Rhizobium* and their hosts by most of species which leads to inductive regulation *nod*.

Pathogenic microorganisms causing damage to the host in various ways, including: production of phytoxins; out-cell production of polysaccharides; degradation of plant tissues by pectinolythic, proteolythic, and cellulolythic enzymes; or production of phytohormones [Long and Staśkiewicz 1993]. Amazing are the means by which plants protects itself from pathogens, for example: synthesis of anti-bacterial metabolites (phytoalexine); cell walls supported by lignin and cellulose; production of chitinase and gluconase enzymes which can damage cell walls of the pathogen or supersensitivity (HR) [Dixin 1994]. Induction of HR leads to necrosis of cells included in the pathogen invasion [Staśkiewicz 1995].

If the pathogen is recognized by the plant in an early stage of development, chances of limiting the development of the pathogen using the HR method by the plant are greater. Intensive research allowed to identify the resistance genes (R) which allow to recognize the specific race of the pathogen transporting avirulently (avr) genes and inducing HR after the invasion.
SUMMARY

Studies on the interactions between soil, plant and microorganisms are at present the most important studies from the point of view of ecology. Microorganisms have accompanied the Earth for about 4 billion years. They take part in the soil formation processes and origin of the soil physical and chemical properties. Beside mother rock, microorganisms and plants have a decisive influence on its fertility and plant growth and development. Up till now there is a discussion going on the influence of microorganisms on the plants development and growth since they can develop in the sterile conditions if only indispensable nutrients are supplied for them. It appeared that in the conditions of especially impoverished soils, it is not possible to provide the plant with proper conditions without microorganisms. There are also plants that cannot survive without symbionts. These are orchis and other plants. On the other hand plants via their secretions regulate both the quantity and quality of the microorganisms. Plant roots uptake cations and release H+ and HCO3− and organic acids that causes decrease in the pH level of the rhyzoplane by as much as 2.2 units. The decrease in the pH level exerts a big influence on the occurrence of some of the microorganisms in the immediate neighbourhood of plant roots.

It has been found out that microorganisms have an ability to recognize a suitable plant host by means of signal molecules. It is especially true of the microorganisms that live in close association. It was proved that biochemical molecules of changes in both plants and microorganisms can be signal molecules. Bacterial cells or even fungi spores receive these signals. One of the answers to the signals sent can be activation of the vir u gene in Agrobacterium tumefaciens or activation of the nod gene in the case of papilionaceous plants. It appeared that microorganisms can transfer genetic material in the soil not only to other microorganisms but also to the plant. The mechanisms of transfer are various. They are related to the growth rate, resistance to pathogens, environmental conditions and other factors. The present studies have shown that the interaction between the soil, plant and microorganism is far greater than it has been believed so far.

REFERENCES

ALEXANDER M. 1975: Ekologia mikroorganizmów. Warszawa, PWN.


Interaction between soil, plant, and microorganisms


Author’s address: Prof. dr hab. J. Kobus Institute of Soil Science and Plant Cultivation, 24 100 Puławy, str. Czartoryskich 8