Symposia Biologica Hungarica 11

PROCEEDINGS OF THE SYMPOSIUM ON SOIL MICROBIOLOGY

Akadémiai Kiadó, Budapest

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SYMPOSIUM ON SOIL
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Budapest, 16th—20th June, 1970

(Symposia Biologica Hungarica 11)

Edited by
J. SZEGI

The volume contains the material of lectures delivered during the sessions of the Symposium on Soil Microbiology organized by the Hungarian Academy of Sciences and the Hungarian Society of Agricultural Sciences.

Sixty-five papers written by research-workers of some 19 different countries discuss two major themes; the first part deals with the microbiological transformation of plant residues reaching the soil, while the second with the interaction of pesticides useb in agriculture and soil microbes.

The book may be of interest not only to soil microbiologists but to those working in the field of soil science and agricultural chemistry as well as to specialists dealing with soil cultivation and the application of pesticides in agricultural practice.

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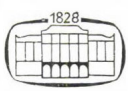

AKADÉMIAI KIADÓ, BUDAPEST 1972

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FOREWORD

The problem of increasing agricultural production is presenting a more and more complicated task for biological sciences. The continuous increase of plant production yield is only possible if we are familiar with the soil; the physical, chemical and biological processes taking place in it, and with the application of appropriate agrotechnical methods by which we can direct these in such a way that the fertility of soils be maintained and constantly increased.

I think we all agree that there is an exceptionally clear relationship between the properties of certain soils and the activity of the microorganisms residing in them. It is thanks to the world renowned soil microbiologists such as Winogradsky, Löhnis, Hiltner, Omeljansky, Stoklasa, Fehér and others that in the first decades of our century the one-sided chemical approach was replaced by a biological one. This means that investigations of the soil with chemical and physical methods can only be successful if we consider the trend of the biological and biochemical processes occurring there, since these react directly or indirectly on the chemical and physical properties.

In other words, with chemical and physical methods we can fix a given state of the soil while with the biological approach we can evaluate the soil in change and dynamics. This factor of course is valid vice versa too. We can become familiar with the role of the physiology of microorganisms in nature only if we study the chemical and physical properties of the soil from which they originate.

It is obvious from this also, that soil microbiology is an inseparable branch of those sciences dealing with the soil whose basic aim is to know the physiological activity of the organisms, residues in the soil and the biochemical changes which are a result of this; moreover the transformations and by establishing the rules to hand them over to practice.

This symposium deals with two such aspects which are regarded as urgent problems for agricultural science not only from a theoretical but also practical point of view.

Let us consider the first problem area, the question of the transformation of plant materials getting into the soil. The constant improving of harvesting agricultural products by machine and with its extension result in the fact that an ever increasing number of plant parts apart from the basic crops remain on the surface of the soil or rather in the soil. Moreover, the improved animal husbandry methods as well as the production of intensive cereal varieties and their machine harvesting result in the

end in the decrease of farm manure. This entails that the nutrient replacement will become an ever increasing task of the fertilization industry.

It is not indifferent for us how the huge plant mass remaining in the soil influences the efficiency of fertilizers and how it affects the carbon cycle which finally is manifest in the quantity and quality of humic materials; also the organic compounds of plants remaining after harvesting can maintain the balance of organic matter content of soil or even perhaps raise it? By clarifying these questions serves to help the agrotechnical methods in their further improvement.

The other problem sphere which is concerned with the interrelationship of pesticides and microorganisms applied in agriculture is just as important. Modern agriculture is ever more widely using the different herbicides, fungicides and insecticides. The chemicals used in the course of plant protection reach the soil partly or wholly and there come in contact with the microscopic organisms residing there. This relationship which is mutual is extremely complicated and complex. On the one hand, the pesticides may inhibit or stimulate the physiological processes of certain microorganism groups, and thus change the direction and pattern of the microbiological processes. On the other hand, in the detoxification of pesticides the microorganisms have a great importance besides the chemical and colloid-chemical factors, because these either indirectly or directly neutralize the pesticides which have accumulated superfluously in the soil. This takes place indirectly in such a way that the pesticides come in contact with the metabolic products of different microorganisms and lose their effect; certain microorganisms can utilize them directly as nutrient source.

The Ministry for Agriculture and Food highly esteems the aims of the Symposium because it has set as its central task the problem of increasing soil fertility which actually serves to raise the food supply of mankind. Irrespective of our believes, we consider it not only an honourable task but a duty to mankind.

G. Soós
The First Deputy of the
Minister for Agriculture and Food,
President of the Hungarian
Society of Agricultural
Sciences

FOREWORD

The first international Hungarian Symposium on Soil Microbiology aimed to discuss very real problems. The microorganisms influence basically the formation of the soil fertility in the course of the transformation of soil organic materials. The interaction between soil microorganisms and herbicides is such a subject which on the effect of using chemical materials is one of the new factors of the agriculture of our age—chemicalization which makes it so real.

The fertile soils had many such new factors during recent years, which earlier did not exist. Among them particular attention should be paid to the changed composition of the organic materials introduced into the soil, the use of fertilizers in large amounts, the general use of pesticides. The importance of the last is well illustrated by the following data: 120 kg pure active ingredient fertilizers are applied yearly nowadays for 1 hectar of agriculturally cultivated territory. The amount of pesticides used in agriculture has increased almost tenfold in one decade. Besides quantitative data that qualitative index is interesting, according to which the number of compounds used as active ingredients of the different fertilizers and pesticides surpasses five hundred.

There is no doubt, that such great changes of which the majority directly affect the soil, result in such a chain of microbiological interactions, whose study and evaluation is not only of scientific but also of general interest.

As it is well known the defense of the biosphere becomes day after day one of the central scientific programs of our age. The scientific organs and scientists of all countries have worked out concrete research themes and large-scale international cooperation and collaboration has been realized.

We have been preparing research plans for the future in the Hungarian Academy of Sciences too. In all likelihood the defense of the biosphere will be such a research program in Hungary for which important material and intellectual power will be concentrated. The research connected with the biosphere will have complex features which will require the cooperation of many branches of science—agricultural, medical, biological, hydro-

logical and sociological respectively—that is, the bringing into harmony basic, applied and developing research.

As regards maintaining and increasing soil fertility, and decreasing the harmful side effects of the use of chemicals has a great role and importance.

We expect the greatest help from the soil microbiologists that they may be able to predict changes and to solve the problem of directing and regulating the physiological processes taking place in the soil.

I. Láng

Deputy Secretary of the
Hungarian Academy of
Sciences

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EDITOR'S PREFACE

It was the first occasion in Hungary that an international scientific symposium was organized in the field of soil microbiology. The programme of the symposium was arranged so that both theoretical and practical problems would be discussed. This is reflected in the two themes. 1. The role of microorganisms in the transformation of soil organic mater, and 2. Interactions between herbicides and microorganisms.

We think that our symposium has achieved its aim. The scientific results presented at it and the discussions conducted there will help the further work of the participants. The personal connections formed at the symposium have strengthened the international cooperation of soil microbiologists.

We should like to express our thanks to the Hungarian Academy of Sciences, Ministry of Agriculture and Food as well as the Hungarian Association of Agricultural Sciences for the extensive moral and financial help, which they gave in organizing the symposium. We are grateful to Professor E. N. Mishustin and Professor K. Domsch who, with the presentation of their high level stimulative introductory lectures have raised the scientific standard of the symposium, furthermore to foreign colleagues taking part in the symposium and hoping they have felt well in our country. We are much obliged for the active support of all participants of the symposium. Last but not least, thanks are due to the untiring cooperation of the colleagues in the Organizing Comittee and others who, inspite of the difficulties, ensured the successful organization and smooth running of the symposium.

Budapest, 1970

J. Szegi
The President of the
Organizing Committee

SECTION I

THE ROLE OF MICROORGANISMS IN THE TRANSFORMATION OF SOIL ORGANIC MATTER

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POTENTIAL AND EFFECTIVE SOIL FERTILITY AS RELATED TO PLANT REMAINS*

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The richness of organic matter in the soil has always been associated with fertility. It is only too natural therefore that the properties of soil organic compounds have been studied with such attention from the very beginning of natural history as a sicence.

It will be recalled that at early stages, serious blunders were made in assessing the role of organic compounds in soil fertility. There appeared Thaer's "humus" theory of nutrition, according to which, humus was a direct source of plant nutrition. For a score of years or so, the "humus" theory was universally accepted until Boussingault and Liebig the founders of the mineral theory of plant nutrition, published their works. Yet, even this new approach sometimes led to extremes and all sorts of erroneous notions as to the role of humus.

A large series of subsequent researches in the last century, the most prominent of them — by Schlösing, Kostychev, Dokuchaev, Deherain and Demussi — made it possible to establish the crucial importance of humus in imparting valuable agronomical characteristics to the soil. It was shown that humus was a source of plant nutrients (above all nitrogen) passing during decomposition into available forms. Humus provides the medium with a combination of extremely valuable nutrients and hence potential soil fertility to build up. Besides, humus shares in the building of a soil structure absolutely essential for a most favourable air and water regime in the soil. And finally, by forming complex compounds with many soil mineral components, humus makes these components available to plants.

In recent years, Flaig (1968) and Christeva (1953) succeeded in showing that individual fractions of humus compounds or their decomposition products stimulated plant growth.

This conclusion fully correlates with the modern view of organic and organo-mineral fertilizers as the most effective suppliers of nutrition to plants.

Today, there remains little or no doubt about soil humus being extremely useful and about the importance of not only its preservation, but also its accumulation in cultivated soils. Indeed, according to Kononova (1963), soils under cultivation tend to lose humus quite appreciably, the agronomical characteristics of their arable layer thus get worse. In 12—13 years of cultivation, virgin soils lose humus at the following rates: up to 40% in

* Introductory lecture of Section I.

the podzolic zone; 7% in the chernozem zone; 70% in the serozem (gray soil) zone.

Especially acute are the losses of humus in the soil on application of mineral fertilizers sharply stimulating the mineralizing activities of soil microorganisms as reported by Mishustin and Nikitin (1961). Now when chemical fertilizers have been increasingly used in agriculture, the problem of supplementing the lost humus becomes particularly prominent, the more so, as the usual practice is to apply physiologically acid mineral fertilizers which impart acidity to the soil giving rise to adverse phenomena. These phenomena have a tendency to expand with time, and especially rapidly, in low-humus soils. Sometimes toxic aluminium salts become soluble, phytopathogenic fungi multiply faster, etc.

The increasing application of mineral fertilizers calls for considering the problem of soil humus from different angles.

The patterns on which humic substances build up under natural conditions are now quite clear. Being unable to analyse literature available on this problem, we shall limit the present discussion to certain generalizations. Rodin and Bazilevich (1965) give us a general idea of the quantities of organic matter and chemical elements contained in the litterfall of plant communities of different climatic zones. These quantities grow in direction from north to south. However, the accumulation of humus in the soils of different zones fails to yield an exponential curve in view of differences in the activity energy of microorganisms from zone to zone. These differences are also responsible for the non-uniformity of the synthesis-decomposition process of soil humus. According to Kononova, in the north, where the number of microorganisms is low and their activity weak, humus is produced in low amounts. Its slow accumulation in the south is determined on the contrary, by too much dispatch with microorganisms destroy plant remnants. The accumulation of humus reaches a maximum somewhere in-between (chernozem soils).

Since the activity of microorganisms is mainly determined by hydrothermal conditions, it is natural enough that Volobuiev (1968) obtained a rather descriptive formula for the relationship between the rate of humus accumulation and the hydrofactor value.

The hydrofactor (Hf) indicates changes in the moisture conditions with different ratios between precipitations (P) and the mean annual temperature (T). Hydrofactor values are calculated by the empiric formula:

$$Hf = 43.2 \lg P - T .$$

High-humus soils are characterized by average hydrofactor values varying from 105 to 112. Thick chernozems have a hydrofactor value of 110. With lower or higher hydrofactor values, the humus content sharply drops.

The soil fertility built up naturally by prolonged cultivation should not be allowed to decrease. Progressive crop-rotation systems are arranged so that each of their components increases soil fertility instead of decreasing it. Lenin wrote that the law of diminishing soil fertility failed to apply whenever technology was making progress and the modes of productivity were transforming.

A very great role in replenishing soil fertility, and humus in particular, is played by perennial grasses (especially legumes). However, large masses

of organic matter in the form of grain and straw of cereal crops are annually removed from the field. Only a smaller part of the removed compounds is returned into the soil in the form of manure, their greater part being lost for ever.

In the USSR, where some 120,000,000 hectares are under cereals, the total yield of straw is at least equal to 250,000,000 tons. At rough estimates, some 100,000,000—110,000,000 tons of this total are not used being mainly burnt up at the place of harvesting, because the transportation of straw from field costs too much, making roughly 50-60% of the total cost of harvesting. The preparation of artificial manure from the straw is also expensive.

In view of this, it would be the most economical to use straw remnants right on the spot as organic fertilizer and some such attempts were indeed made by Russian and foreign researchers. A number of dissertations (Erofeev and Vostrov 1964; Vostrov 1963; Golod 1967; Sinkha 1969, etc.) review literature on this problem in considerable detail and here it will be discussed only briefly.

The first experiments using straw as a fertilizer were staged with a certain measure of success in Russia by Kashirsky (1900) and Kaluzhsky (1906). Later on, Sabinin and Vyalovsky used cotton remnants as a fertilizer for cotton. The experiments with straw remnants were continued by others with variable success (Fedorov 1952; Romashkevich 1966, etc.).

At Rothamstead Station in Britain, straw was tested as a fertilizer by Thornton (1929), and others.

At Versailles Station in France, much experimentation with straw was done by Simon (1959). Later works were summarized by Wicke (1967) and Kühn and Longe (1969). A great deal of attention is given to straw as a possible fertilizer in Australia.

The experiments so far performed provide grounds for a statement that straw remnants possessing a wide C/N ratio on being applied to soil will immobilise nitrogen. Therefore, their application under cereals and other crops not fixing molecular nitrogen should be accompanied by applying mineral nitrogen in an approximate dose of 5—7 kg per ton of straw. Under legumes, straw can be applied without mineral nitrogen, since this family of plants satisfies its demand by fixing N_2 from the air. The above requirements being met, straw fertilizers usually give good results and sometimes increase the yield of farm crops. At the same time, the results from their application may be poor often in the absence of devices for the desintegration of straw before its incorporation into the soil.

In view of the pratical importance of the problem under discussion, the author of this paper and others subjected it to experimental verification on the assumption that the arable layer was heterogeneous. The experiments staged in the laboratory showed beyond doubt that under normal moisture rapid mobilising processes in the upper portion of the arable layer proceed with much greater energy. The upper arable layer produces more carbon dioxide and nitrates, more vigorously destroys cellulose and the process of humus formation in it proceeds with more vigour as well. In the final analysis, the soil of that portion has a higher potential fertility.

It follows from the above that the agronomical effect on the application of straw remnants at different depths should be far from uniform. This

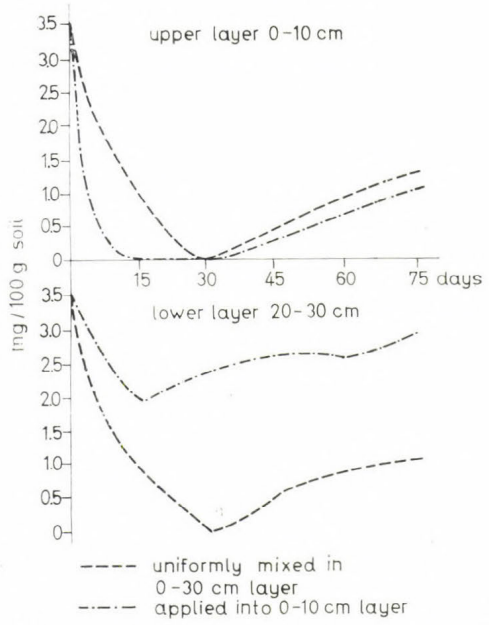
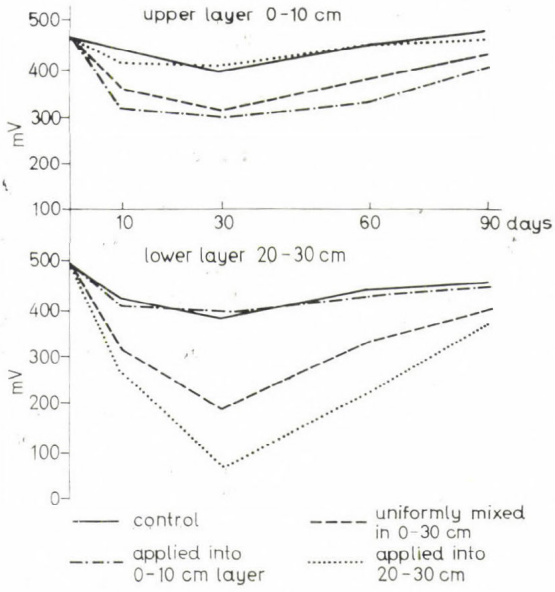


Fig. 1. Dynamics of nitrate nitrogen on application of straw into the soil

Fig. 2. Dynamics of oxidation-reduction potential on application of straw into the soil



supposition was verified by vegetation experiments using podzolic soil from a test plot of the Timiryazev Agricultural Academy near Moscow. Desintegrated wheat straw (into cuttings, about 1 cm long) was applied into vessels holding 8 kg of soil each (35 g per vessel, which corresponds to 10.5 tons per hectare). In the first variant, the straw was applied into the 0—10 cm layer, while in the second, it was uniformly mixed throughout the soil mass in the vessel. The moisture content of the soil was set at 60% of the maximum moisture capacity. The experiment was held at a temperature of 20—25 °C.

The application of straw caused a sharp rise in the activity of microorganisms in the 0—10 cm layer. It will be recalled that even such stable compounds as cellulose and lignin cause the activation of soil microflora (Szegi 1961, Haider and Domsch 1968). Omitting the analysis of the dynamics of microorganisms, we shall propose that their propagation led to the immobilization of nitrogen (Fig. 1). In the lower layer (20—30 cm), appreciable amounts of nitrogen got fixed only on the application of straw.

In the next experiment, conducted under analogous conditions, the oxidation-reduction potential of the soil on different applications of straw was measured, on application:

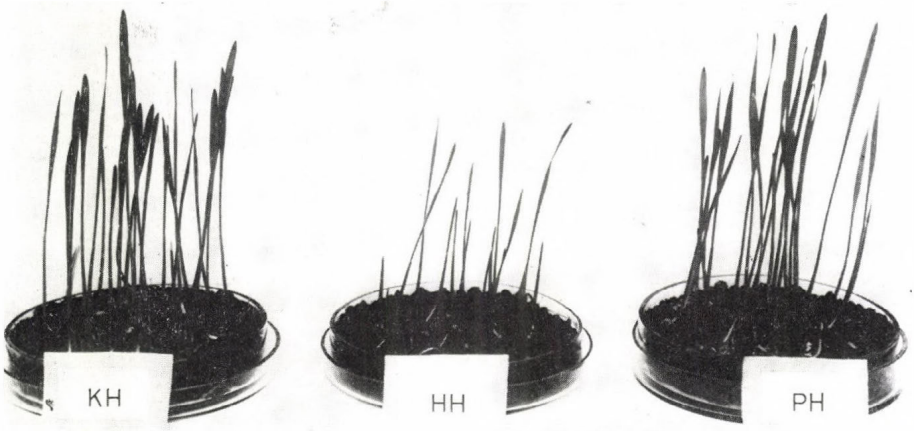


Fig. 3. Germination of wheat in different layers of the test soil; KH-control (no straw applied), HH-soil from the lower layer of the vessel (20—30 cm) on application of straw, PH-soil from the upper layer of the vessel in which straw was incorporated uniformly throughout the soil mass (0—30 cm)

a) into the upper 10 cm;
b) into the entire soil mass;
c) into the 20—30 cm layer.

As it follows from Fig. 2 the application of straw into the upper layer resulted in a somewhat lower oxidation-reduction potential. Approximately in two months, it dropped to 300—330 millivolts. On the application of straw into the lower layer, sharply reduced conditions manifested themselves. The oxidation-reduction potential dropped to 100—200 millivolts for quite a long time.

On the decomposition of straw in the soil, products toxic to plants could have accumulated in the soil. In order to verify this supposition, soil samples were taken from different depths from a vessel into which straw had been applied throughout the entire mass of soil following incubation. The soil was sown with wheat. As shown in Fig. 3, the upper layer was not toxic being the same as the control, whereas on thes oil of the lower layer, the plants were obviously suppressed. The soil was the most toxic after the first 14—20 days of soil incubating with straw.

On increasing straw doses, soil toxicity naturally rose. The inhibiting properties were recorded even in the upper horizon.

Figure 4 shows the effect of water extracts from a soil uniformly fertilized with straw at a rate of 120 g per vessel (60 tons per hectare). Under optimum moisture conditions, the soil was subjected to incubation for 2 weeks at a temperature of 20—25°C. The water extract was prepared by proportioning 1 part of soil with 2 parts of water. Wheat seeds placed on filter paper were moistened with filtered extract. It turned out that an extract from the upper horizon somewhat delayed the germination of the seeds, while that from the lower horizon suppressed it completely.

The determination of decomposition products from plant remnants was made by chromatography. Compounds were determined in an ether

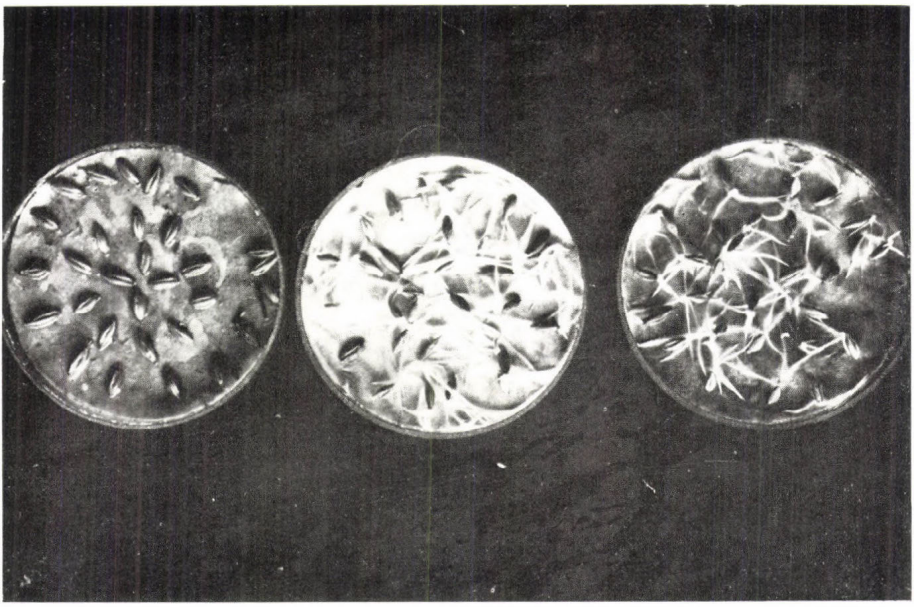


Fig. 4. Effect of extracts from different soil layers fertilized with straw: 1 — seeds treated with an extract from the lower soil layer fertilized with straw; 2 — control (seeds treated with water); 3 — seeds treated with an extract from the upper layer fertilized with straw

extract, in a water extract, and then Forsyth's procedure (1947) of column chromatography on animal charcoal was used in order to partition carbohydrates, phenolic compounds and fulvic acids. In an alkaline extract, the content of humic acid was determined. Most of the testing was done on vegetable material labelled with carbon (^{14}C). The test material which made up 2% of soil by weight was incubated at a temperature of 30°C for 3 months. The soil was moistened to 60% of the maximum water-holding capacity.

Among the substances recorded were volatile acids (acetic and butyric), several more organic acids (tartaric, malic, oxalic, etc.), phenolic-type substances, carbohydrates, amino acids and other organic compounds. In the alkaline extract, 19—27% of ^{14}C occurred in the form of humic acid and 8—14% in the form of fulvic acids. In the water extract, some 5—7% of ^{14}C occurred in the form of fulvic acids. During the experiments 38—55% or organic plant remnants were humified.

During the experiments, the volatile acids were viewed with particular attention. Mostly accumulating in the lower soil layer, their accumulation was directly proportional to the manifestations of toxicosis. Therefore the volatile acids were regarded as possible suppressors of plants.

Humus compounds were also studied in more detail. It turned out that they accumulated most vigorously in the upper, better aerated soil layer. This pattern was more vividly revealed in a longer test using sand as substrate (lasting one year). An alkaline extract from the upper layer yielded many more dark coloured compounds than that from the lower layer.

The data collected indicate convincingly enough that the decomposition of plant residues does not follow the same pattern at various depths of the arable layer.

Water-soluble humus compounds represented by fulvic acids were interesting as possible solvents of low active soil mineral compounds since they are capable of forming chelate compounds (Vishnyakov 1935, Wiesemüller 1965).

In order to reproduce the process of solution of mineral compounds by fulvic acids, $FePO_4$ (Fe and P being radioactive) was acted upon with a fulvic acid solution. These tests confirmed the conversion of phosphorus and iron into solution, the dissolution of phosphates became more intensive with increasing fulvic acid concentration.

The interaction between fulvic acids and phosphates was examined by electrophoresis in a reflected ultra-violet light. The electrophoretograms served to obtain radioautographs which showed the distribution of labelled phosphorus over the former. And finally, densitograms were obtained on X-ray film on the basis of the radioautographs and electrophoretograms. Using this method, it was possible to study both the joint and separate movements of labelled phosphorus in the electric field.

On the interaction of ash-free preparations of humus components with the phosphate (^{32}P), the zones of phosphorus and organic matter moved separately. Consequently, no stable non-dissociating compounds of phosphorus with organic matter were formed in this case.

In the presence of the fulvic acids of aluminium or iron, in the molecules there was a joint movement of phosphorus and organic matter recorded on the electrophoretograms. Consequently, non-dissociating compounds were formed in this case.

Next, complex compounds of fulvic acids with iron phosphate were obtained. Their C, P and Fe were radioactive labelled isotopes. These complex compounds were taken up readily enough by young plant roots and took only a little more time to pass to the aerial parts than soluble phosphorus compounds in the form of ions. There is a possibility of phosphoro-organic compounds getting hydrolyzed while passing through the plant root, yet the transformation of phosphorus into a soluble form by fulvic acids will at least serve to facilitate the assimilation of these compounds.

The organic substances applied into the soil in the form of straw give rise there, to a vigorous propagation of saprophytic microorganisms, some of them fixers of molecular nitrogen. In view of soluble nitrogen compounds being fixed, the latter microorganisms must be very active indeed. The tests with $^{15}N_2$ show that 1 g of cellulose introduced into the soil the microorganisms living in it fixed on the average about 5 mg of N_2. This rate of fixation may considerably vary with varying conditions. This build-up of nitrogen may be of definite practical value.

Thus, as straw gets decomposed in the soil, simultaneously with the formation of products toxic to plants, processes which may serve to increase yields of crops also proceed. With due regard to when these processes are under way, it should be possible to take advantage of such periods to promote plant growth.

Next, a series of experiments were staged with a view to determining the effect of straw application on the yields of farm crops. According to preliminary tests, when applied shortly before sowing, straw adversely affects plant growth. In a few months of incubation, the decomposition products of straw, even on application deep into the soil, cease to adversely affect even cereals. This is illustrated by the following experiment with oats using soddy-podzolic soil from a test plot of the Timiryazev Agricultural Academy. 36 g of disintegrated straw of winter wheat were applied either in the upper layer of soil (0—10 cm) or in the lower portion of a test vessel (20—30 cm). The test vessel contained 8 kg of soil. In the first variant, straw was applied in spring just before the sowing of oats, in the second — well in advance in the autumn, six months before the spring sowing. In winter, second-variant vessels were kept in a basement with a positive air temperature. The moisture content was constantly maintained at 60% of the maximum water capacity. As clear from Table 1 which indicated the dry weight of the yield, the incubation of straw for six months was practically enough to remove its toxicity.

Table 1

Relation between the time of straw application and the yield of oats (g per vessel)

Variant of experiment	Weight of aerial mass	Weight of grain
1. Straw applied just before sowing		
in 0—10 cm layer	5.8 ± 0.24	2.33 ± 0.05
in 20—30 cm layer	2.78 ± 0.09	0
2. Straw applied six months before sowing		
in 0—10 cm layer	6.57 ± 0.28	3.0 ± 0.28
in 20—30 cm layer	6.80 ± 0.48	3.33 ± 0.19
3. Control (no straw applied)	7.73 ± 0.24	3.76 ± 0.08

Here are the results of experiments with legumes.

Only a few of a large series of experiments are discussed below. All the experiments were conducted using podzolic soil from a test plot of the Timiryazev Agricultural Academy under the same conditions as the above experiment with oats. Straw was applied six months before sowing.

Just before sowing, the seeds of legumes were inoculated with pure cultures of appropriate nodule bacteria. Table 2 shows an experiment with vetch which gave a higher yield owing to the surface application of straw. The yield of grain increased by up to 40%. There were 18 vetch plants per vessel.

The experiment was aimed to study the after-effect of fertilization of vetch with straw on the yield of oats. After harvesting vetch, the soil was loosened with a metal rod to be left until the next spring to be sowed with oats (10 plants per vessel). As follows from Table 3, in all the variants the increase in the yield was considerable, but the best result was obtained

Table 2

Effect of straw application on the yields of vetch (g per vessel)

Variant of experiment	Weight of aerial mass	Weight of grain
Control	16.10 ± 0.24	7.35 ± 0.21
Straw applied uniformly throughout soil mass	18.50 ± 0.17	9.01 ± 0.19
Straw applied in upper layer (0—10 cm)	20.90 ± 0.20	10.35 ± 0.25
Straw applied in lower layer (20—30 cm)	17.70 ± 0.15	8.73 ± 0.19

Note: In this and subsequent tables above-ground mass and grain yield are given in terms of dry weight.

on the surface applicati on ofstraw, with the yield of grain increasing by 30%. Annual lupine well responded to the surface application of straw by increasing the yield of green mass (in terms of dry weight) by 25% (Table 4). There were four lupine plants per vessel.

The experiment with beans also confirmed the high effectiveness of straw application into the upper soil layer (Table 5). There were eight plants per vessel.

Table 3

After-effect of straw application on the yields of oats (g per vessel)

Variant of experiment	Weight of aerial mass	Weight of grain
Control	16.96	7.13
Straw applied uniformly throughout soil mass	21.80	9.05
Straw applied in upper layer (0—10 cm)	22.50	9.37
Straw applied in lower layer (20—30 cm)	18.70	8.22

Table 4

Effect of straw application on the yields of annual lupin (g per vessel)

Variant of experiment	Weight of aerial mass
Control	8.56 ± 0.1
Straw applied in upper layer (0—10 cm)	10.75 ± 0.25
Straw applied in lower layer (20—30 cm)	7.12 ± 0.75

Table 5

Effect of straw application on the yields of beans (g per vessel)

Variant of experiment	Weight of aerial mass	Weight of grain
Control	10.16 ± 0.34	1.15 ± 0.03
Straw applied uniformly throughout soil mass	9.27 ± 0.21	1.29 ± 0.09
Straw applied in upper 0—10 cm layer	11.58 ± 0.24	1.49 ± 0.09
Straw applied in lower 20—30 cm layer	9.90 ± 0.28	1.29 ± 0.08

The best results were obtained on the application of straw in the upper soil layer, with the yield of grain increasing by 30%.

In the above experiments using soil, it was difficult to calculate the nitrogen balance, and therefore for certain legumes soil was replaced by sand as substrate. Hellrigel's mixture containing the initial dose of nitrogen was applied into washed quartz sand. The initial dose was usually equal to 35 mg of nitrogen per vessel in the form of NH_4NO_3. For beans, the dose of nitrogen was doubled. Before sowing, all the seeds were inoculated with pure cultures of nodule bacteria.

Below, the data collected in some of the experiments and a table summing up nitrogen increment per vessel can be seen. The description of the experiment is begun with vetch (see Table 6). The same as for the experiments using soil there were 18 plants per vessel.

Table 6

Effect of straw application on the yield of vetch in an experiment using sand as substrate (g per vessel)

Variant of experiment	Weight of aerial mass	Weight of grain
Control	14.59 ± 0.38	4.14 ± 0.29
Straw applied uniformly throughout soil mass	23.3 ± 0.36	11.67 ± 0.47
Straw applied in upper layer (0—10 cm)	18.78 ± 0.35	8.89 ± 0.36
Straw applied in lower layer (20—30 cm)	15.73 ± 0.43	7.72 ± 0.34

In this series, the best result was obtained on uniform application of straw throughout the sand mass. But even in other variants, increments to the yield of grain exceeded the control by at least 2—3 times.

Lupine crops responded well to straw application. As it is clear from Table 7, even though the surface application of straw was more effective, the fertilization of the lower layer of the substrate gave a considerable increment of the yield. In this experiment, there were four plants per vessel.

Table 7

Effect of straw application on the yield of lupine in an experiment using sand as substrate (g per vessel)

Variant of experiment	Weight of aerial mass
Control	4.87 ± 0.39
Straw applied in upper (0—10 cm) soil layer	10.43 ± 0.74
Straw applied in lower (20—30 cm) soil layer	7.25 ± 0.43

An experiment similar to the above was also performed with beans. There were eight plants per vessel. See Table 8 for results.

Table 8

Effect of straw application on the yield of beans in an experiment using sand as substrate (g per vessel)

Variants of experiment	Weight of aerial mass	Weight of grain
Control	7.28 ± 0.26	0.15 ± 0.01
Straw applied uniformly throughout soil mass	10.16 ± 0.24	1.14 ± 0.08
Straw applied in upper soil layer (0—10 cm)	12.4 ± 0.27	1.87 ± 0.07
Straw applied in lower soil layer (20—30 cm)	11.9 ± 0.18	1.29 ± 0.04

In a nitrogen-deficient medium, the application of straw had a favourable effect irrespective of application techniques in use. And yet, the best effect was obtained on the surface application of straw, with the yield of grain going up 12-fold.

Both in the above and other experiments using sand as substrate, the nitrogen balance was calculated.

Table 9 gives the data of increment vs. total nitrogen content in the soil-plant system in mg per vessel.

As it is clear from the above, straw application sharply increased the nitrogen-fixing activity of legumes. In the majority of instances, the surface application of straw gave better results. The only exception was vetch which somewhat better assimilated molecular nitrogen on the uniform application of straw.

Table 9

Effect of straw fertilizer on the fixation of molecular nitrogen by legumes (mg per vessel)

Variants of experiment	Vetch	Lupine	Beans
Control (no straw is applied)	398.3	140.1	99.1
Straw applied uniformly throughout soil mass	617.6	—	190.5
Straw applied in upper soil layer (0 – 10 cm)	535.5	320.2	265.5
Straw applied in lower soil layer (20 – 30 cm)	408.7	210.8	229.1

According to rough calculations, nearly 5 mg of molecular nitrogen were assimilated per gram of straw, usually on the surface application of straw, other conditions being favourable. Consequently, on the fertilization of one hectare of soil with 4 – 5 tons of straw, it should be possible to gain nearly 20 – 25 kg of nitrogen through the intensification of nitrogen assimilation.

Of the whole series of investigations on considering the problem of fertilizing effect of straw application on cereals we shall discuss only two. A vegetation experiment was carried out under the same conditions as for legumes and on the same soil (Timiryazev Agricultural Academy). Straw cuttings were applied to a depth of 0 – 7 cm before sowing oats (10 plants per vessel). At different stages of the experiment, different amounts of nitrogen fertilizer were applied in the form of $(NH_4)_2SO_4$, while phosphorus and potassium in Hellrigel's dose. When applied in low doses, straw inhibited the growth of oats, while its increased doses stimulated its growth (Table 10). This is confirmed by other researchers such as Simon (1959).

Table 10

Effect of straw application on the yields of oats (g per vessel)

Dose of nitrogen fertilizer per vessel (in terms of N, g)	Control (no straw applied)	Straw applied
0.1	21.84	11.68
0.5	26.77	25.43
1.5	29.14	31.82
3.0	26.41	30.36

On microchecks in the Krasnodar Border Region, rice crops were fertilized with rice straw (4 tons per hectare). For comparison, approximately the same amount of alfalfa was applied as green manure. The fertilizers were ploughed in already in autumn. During the vegetation period $(NH_4)_2SO_4$ was applied in a dose of 100 kg of nitrogen per hectare.

The application of straw practically gave the same results as the application of alfalfa (Table 11).

The experiments like this have now been staged for three years and with analogous results. On addition of a nitrogen fertilizer, the application of straw resulted in a 20 – 50% increase in the yields of rice.

Table 11

Yields of rice on microchecks on application of different organic fertilizers

Variants of experiment	Yields	
	centners/ha	%
Control	38.8	100
Alfalfa ploughed in 0—20 cm layer	58.4	150
Straw ploughed in 0—7 cm layer	55.0	142

This successful experiment suggests that, under certain conditions, straw may be used to fertilize cereals as well.

In conclusion, let us discuss the problem of raising the availability of phosphorus compounds to plants by applying straw fertilizers. The experiments were performed with several phosphates poorly available to plants such as apatite, $FePO_4$ and $AlPO_4$. In some of the experiments, use was made of compounds containing radiophosphorus. We shall limit the present discussion to experiments with apatite and $FePO_4$.

A phosphorus-low podzolic forest soil from a test plot of Timiryazev Agricultural Academy containing 15.4 mg of available P_2O_5 per 100 g was used in the experiment. It was limed with regard to the maximum hydrolytic acidity. Nitrogen in the NH_4NO_3 form was applied in a dose of 150 mg per 8 kg vessel, and P_2O_5 (apatite or $FePO_4$) in a dose of 50 mg per 100 g of soil. 50 g of straw cuttings were applied in the upper layer 1.5 months before sowing. The first crop, vetch, was sown at a rate of 18 plants per vessel, and the second, oats, 10 plants per vessel. The data collected signify that straw application facilitates the gradual conversion of low-active phosphates into an available form. In the second year, on the soil fertilized with straw the plants assimilate much more phosphorus from apatite and $FePO_4$ (Tables 12 and 13).

Table 12

Effect of straw application on the availability of phosphorus in apatite (all calculations made per vessel)

Variants of experiment	Yields of crops (g)		Uptake of P_2O_5 (mg)	
	1st year: aerial mass of vetch	2nd year: grain of oats	1st year: vetch	2nd year: oats
Control	9.38	4.13	55	38
Oats straw applied	8.03	7.19	58	62
Rice straw applied	7.26	7.27	52	63

The fact that straw application failed to produce any positive effect on vetch yields may be explained by the straw being applied before sowing in an increased dose. As a result, by the time of sowing, the soil still retained toxins. At any rate, a year later its positive effect on the uptake of phosphorus manifested itself obviously enough. Similar results were obtained for $FePO_4$.

Table 13

Effect of straw application on the availability of phosphorus in $FePO_4$ (all calculations made per vessel)

Variants of experiment	Yield of crops (g)		Uptake of P_2O_5 (mg)	
	1st year: aerial mass of vetch	2nd year: grain of oats	1st year: vetch	2nd year: oats
Control	9.81	4.6	61	34
Oats straw applied	9.70	8.03	68	66
Rice straw applied	8.85	7.26	54	68

The experiments with labelled $FePO_4$ and $AlPO_4$ showed that the application of straw not only facilitated the uptake of ^{32}P but also the phosphorus of other compounds thereof. Thus, the application of straw appreciably increased the availability of total phosphorus in the soil.

ABSTRACT

1. The straw applied into the soil undergoes different transformation at different depths. In the upper layer, organic compounds get mineralized more quickly and toxic compounds are accumulated to a lesser degree (mainly volatile acids).

In view of this, it is advisable to incorporate the straw fertilizer into the upper portion of the arable layer.

2. It is almost certain that on the mineralization of straw the soluble forms of nitrogen get biologically fixed. Therefore the application of straw under cereals has to be accompanied by the application of nitrogen-containing mineral fertilizers. At the same time, legumes capable of fixing molecular nitrogen in symbiosis with bacteria have enough nitrogen after the application of straw fertilizer.

3. The straw incorporated into the soil promotes the activity of free living nitrogen-fixers and the conversion into solution of low-active nutrients, a conversion caused by the acid products of cellulose decomposition and the solvent action of chelate-producing humus compounds.

4. The straw incorporated into the soil well before sowing appreciably increases the yields of legumes and stimulates the accumulation of fixed nitrogen by legumes.

The application of straw together with nitrogen fertilizers may appreciably increase the yields of grain crops.

As indicated earlier in this abstract, straw fertilizers are best applied in the upper portion of the arable layer.

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EFFECT OF PLANT COVER AND SOIL CULTIVATION ON THE NUMBER OF MICROORGANISMS AND CONTENT OF ORGANIC SUBSTANCES IN THE SOIL

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It is generally recognized that higher plants constitute the main source of organic nutrition of the soil. During the vegetation period they enrich the soil with organic substances via root excretions, dead root hairs and fine roots; at the end of the vegetation period, through dead roots, crop residues, manure and litter. In both cases plants have a considerable effect on the quantitative and qualitative composition of microflora, particularly in the near-surface layer of the soil. Moreover, plants also have an indirect effect on soil microflora through changes in physical, chemical and physico-chemical properties of the rhizosphere.

The species and variety composition of plant, their development, spacing, soil qualities, climatic conditions, fertilizers and soil cultivation also contribute largely to the content of organic substances and the qualitative and quantitative composition of microflora in the soil. The effect of these factors may be positive or negative.

According to data in the literature, root excretions made the greatest contribution to the increase in the number of microbes and to the control of their qualitative composition in the soil. Ordinarily the rhizosphere contains from 2 to 5 times as many microorganisms as are present outside the rhizosphere; some investigators report figures which are hundreds and thousands of times larger. From this it would be expected that all uncultivated soils (virgin soils, long fallow, soils under forests) would contain more microorganisms than cultivated ones (bare fallow, fields occupied by intertilled and cereal crops) since the former have been infiltrated with exosmotic roots for a longer time and are better provided with plant residues. In fact, the situation is quite the reverse, as is shown by the work of Mishustin (1956). He reports that 1 g of cultivated soil always contains more microbes than 1 g of uncultivated soil (Table 1).

An examination of the literature has failed to provide answers to the questions: what causes an increase in the number of microbes in soils under cultivation, or what inhibits the development of microorganisms in uncultivated soils. Therefore a number of investigations have been carried out by us for the purpose of clarifying them. The results will be given briefly below.

For three years we have continuously studied (Samtsevich 1953) microflora dynamics and certain agrochemical properties of southern chernozem under identical soil and climatic conditions. Observations were made at stations under closed 72-, 40- and 22-year-old oak stands, on the one hand, and under bare fallow on the open steppe and in fields enclosed between rows of

Table 1

Number of microorganisms in the different soils of the Soviet Union (thousands per/g)

Soils	State of soil	Total number of bacteria including spore formers	Number of spore formers	Actinomyces	Fungi	Total number of microbes
Tundro-gley	Virgin	2040	13	30	70	2140
	cultivated	4750	27	84	36	4870
Turf-podzolic	Virgin	970	130	90	26	1086
	cultivated	1800	430	790	30	2620
Chernozem	Virgin	2300	750	1300	30	3630
	cultivated	2940	1000	1570	23	4533
Chestnut	Virgin	2260	690	1200	22	3482
	cultivated	4540	1680	2100	20	6660
Brown and chernozem	Virgin	2920	770	1550	20	4490
	cultivated	4980	1470	2380	18	7370

trees on the other. Agrochemical data obtained have shown that the soil under forest contains a much larger quantity of organic substances, particularly of the water-soluble fraction and nitrogen, than soil under bare fallow (Table 2). In spite of this the total number of bacteria, their most

Table 2

Organic substance and nitrogen content in southern chernozem. Moisture content under bare fallow and closed oak stands on dry steppe

Depth, cm	Organic substance			Nitrogen, %	Moisture content, %
	Total quantity, %	Loosely bound, %	Water soluble, mg %		
Under bare fallow on the steppe					
0—3	4.41	0.64	19.4	0.23	15.3
5—20	4.24	0.52	22.0	0.22	19.5
30—45	2.56	0.22	17.5	0.11	16.2
Under closed oak stand					
0—3	6.14	0.91	86.8	0.47	25.1
5—20	5.38	0.75	48.4	0.32	16.7
30—45	3.55	0.43	32.0	0.18	14.0

important physiological groups, number of spore formers and anaerobic bacteria, actinomyces and fungi are usually found to be smaller in the case of forest than of bare fallow. The difference in favour of microorganisms in bare fallow soil can be seen especially clearly if the figures are converted to number per unit of total organic substance, and even more so per unit of the water-soluble fraction (Table 3). Over the whole period of observation a maximum of microbes and water-soluble organic substances in the soil under forest and fallow is found in autumn and winter.

Table 3

Number of microorganisms in southern chernozem under bare fallow and under closed oak stands on the southern steppe [in mill/g of organic substance]

Microorganisms	Depth, cm	Under bare fallow			Under forest		
		Number of analyses	Number of microbes		Number of analyses	Number of microbes	
			%/g of total organic substance	per 1 mg per cent water-soluble organic compounds		%/g of total organic substance	per 1 mg per cent water-soluble organic compounds
Total number of bacteria	0— 3	20	35.1	1.8	27	21.9	0.25
	5—20	20	92.6	4.02	27	16.5	0.34
	30—45	20	12.2	0.68	27	9.6	0.3
Spore-forming bacteria	0— 3	19	12.4	0.61	26	6.9	0.079
	5—20	19	10.9	0.49	27	6.8	0.14
	30—45	19	4.2	0.24	27	2.6	0.081
Anaerobic bacteria	0— 3	12	3.0	0.15	19	1.6	0.018
	5—20	12	3.9	0.17	19	2.1	0.043
	30—45	12	1.1	0.062	19	0.9	0.028
Actinomyces	0— 3	16	17.1	0.88	22	23.0	0.26
	5—20	16	23.7	1.07	23	16.4	0.34
	30—45	16	11.1	0.63	23	10.7	0.33
Fungi	0— 3	15	0.7	0.035	20	1.2	0.013
	5—20	16	0.4	0.018	23	0.2	0.004
	30—45	16	0.2	0.011	23	0.1	0.003

To reveal the causes of this inverse relation between the number of microbes and organic-substance content in fallows and soils occupied by plants, it was decided to direct our attention to those characteristics of the soils which constitute their main differences: cultivation (aeration) of the soil and production of root excretions.

Composting of soil samples has shown that when soils under forest are periodically disturbed the number of microbes and content of nitrogenous mineral compounds increases considerably and the quantity of water-soluble organic substances decreases, whereas in bare fallow samples such changes were not found. This allowed us to assume that the cause of microbe inhibition in uncultivated soils was a higher content of toxic volatiles produced by microbes and possibly also by plant roots.

A fairly rapid decrease in the number of microbes in the upper portion of the layer turned over by the plough and a simultaneous increase in the number of microbes in the lower portion of the layer when lifted to the soil surface (although organic substance content is smaller in the latter case) may afford some evidence of the deleterious effect of volatile compounds produced by microbes on the development of the latter in the soil (Table 4). The favourable effect of ploughing under straw to a shallow depth on the development of plants and microbes may be explained in the same way.

Table 4

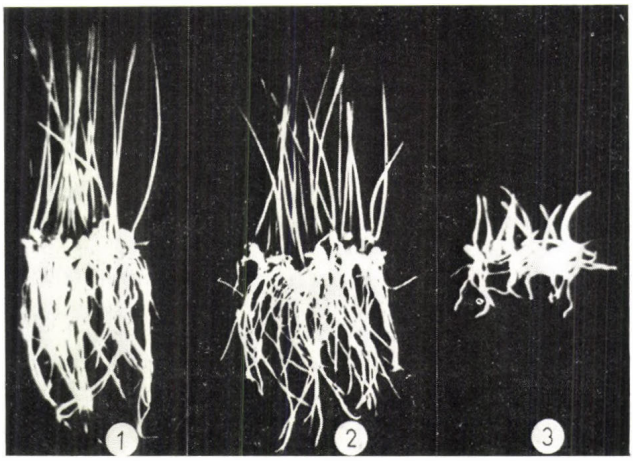
Effect of soil cultivation on number of bacteria in different soil layers (thousand/g)

Depth, cm	Before ploughing		21 days after ploughing			
			turned over		not turned over	
	aerobic	anaerobic and facultative	aerobic	anaerobic and facultative	aerobic	anaerobic and facultative
0— 5	4880	3790	5120	4060	5610	4280
18—25	3730	4240	3680	3570	3830	4320

The positive effect of easily assimilated organic substances on the production of toxic volatile compounds by microbes was experimentally confirmed by us (Samtsevich and Borisova 1963) in special experiments carried out in closed moist chambers. It was found that intensive development of microbes results in the accumulation of a large amount of toxic volatiles (ammonia, butyric acid, hydrogen sulphide, etc.) that affect not only microbe development but also the germination rate of seeds and growth of seedlings, the effect of volatiles being much more pronounced in the air above the soil than in the soil itself (Fig. 1).

Frequently the soil is broken up, the freer it is of toxic volatile metabolites, thus microbes develop better. This may be the explanation of the fact that the largest number of microbes inhabit and most actively manifest themselves in soils under bare fallow and intertilled crops; the number of microorganisms is smaller in the soils under cereal crops, and there are even fewer microbes in soils under perennial grasses, long fallow, virgin lands and forests; plants grow much better on cultivated soils than on un-

Fig. 1. Developed germs of winter wheat. 1) Wet chamber without soil. 2) Composted in wet chamber (kept in incubator) with soil. 3) The same added with 1% sugar



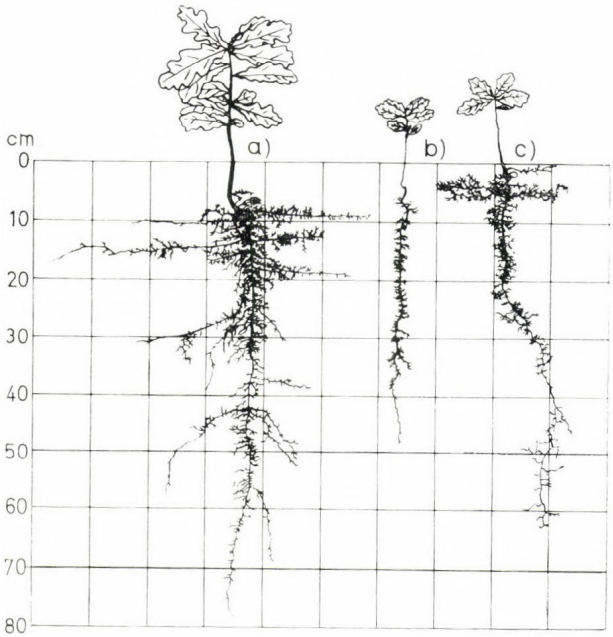


Fig. 2. The growth of oak seedlings and the distribution of their root system in the cultivated and uncultivated soils. *a)* One-year seedling grown with maize covering plant. *b)* The same from spontaneous spearing. *c)* Two-year oak seedlings from spontaneous spearing

cultivated ones, since their root systems are concentrated mainly in the soil layer adjacent to the surface which is better aerated (Fig. 2).

On the basis of the above data we consider that the positive effect of mechanical cultivation of the soil on the vital activity of microbes and plant growth consists not so much in enrichment of the soil by oxygen as in the removal from it of toxic volatiles produced by the microflora. This may be confirmed by the much greater number of microbes in the surface layer of ploughed soils than in their subsurface layers, by the smaller size of bacterial cells in the soil than that of microbes cultivated on dense substrates, etc.

Doyarenko (1926) has shown that "soil air must be recognized as an organic constituent of the soil together with soil solution and the solid phase." Unfortunately the analysis of soils as yet does not involve determination of toxic volatiles.

Root excretions may also be one of the important factors which inhibit the development of microbes in uncultivated soils, although most workers are not in agreement with our position on this question.

Cholodny (1949) was the first to observe the toxicity of actively growing root tips and that they are populated by a large number of soil bacteria. Later this effect was observed by Rempe and Sorokina (1950), Samtsevich (1964), Rovira and McDougall (1967). The phenomenon is attributed to

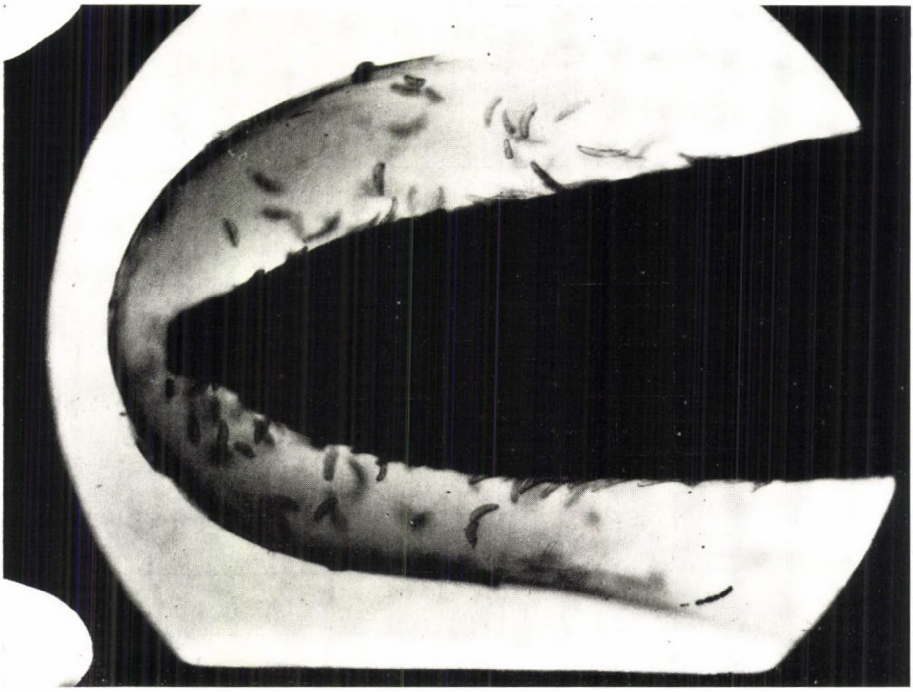


Fig. 3. Gel-like protecting coat from the root of maize which is secreted together with plant cells

the fact that root caps excrete not only sugars and organic acids which are easily assimilated by microbes but also substances which protect corkless roots from microbes that settle on them in large numbers. Our investigations have shown (Samtsevich 1965, 1968) that these substances are concentrated in colourless gel-like excretions of root tips and plant cells sloughed off by them which form a special kind of protective cap around root tips. These latter are not visible under the microscope if they are unstained but can be seen fairly well when stained with aniline dyes, and in maize they can be seen with the naked eye (Fig. 3).

The gel-like substance covering root tips is a high-molecular polysaccharide (pectic substances, hemicellulose); it is not soluble in water, alcohol, ether, HCl and NaOH at room temperature, thus adherence to the roots is facilitated. Vegetative cells sloughed off by root caps, lying in the gel-like substance, retain their vitality for up to two days or more, regardless of sterile conditions (Samtsevich and Mironova 1969). High osmotic pressure in them of up to 8—9 atm. affords evidence of the above statement (Fig. 4). Preliminary calculations have shown that the quantity of gel-like substances in cereal crops per 1 ha. is about the same as of dry substances of very high grain yield. Their role is quite significant and varied both for plants and for soil-forming processes; their main property is probably protection of tender corkless roots being populated by a large number of potentially harmful microorganisms.

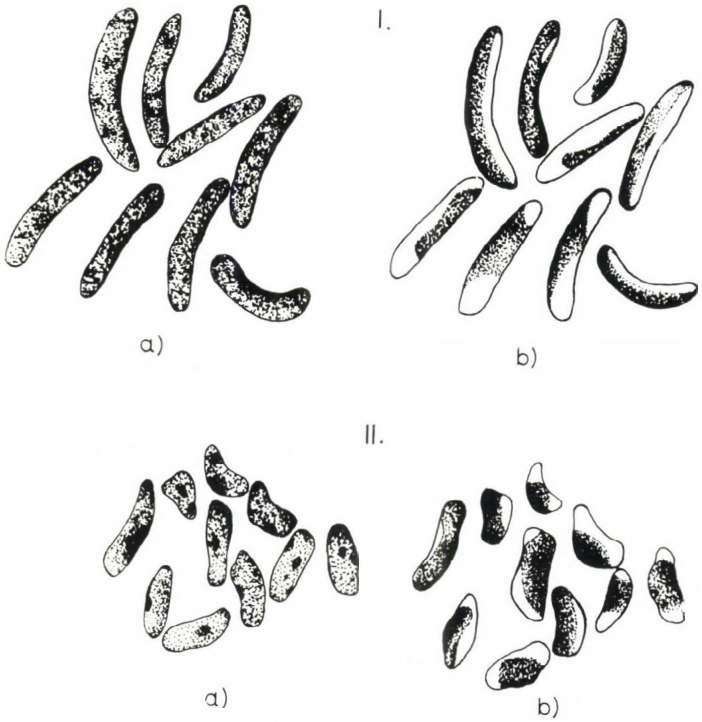


Fig. 4. Cells separated from the root coat of pea (I) and maize (II). *a)* no plasmolysis, *b)* plasmolysis with 0.5 M saccharose solution

Since in perennial plants the total length of roots, vegetation period and mass is much larger than in annual agricultural crops, and the soil under the former is not broken up, it is natural that uncultivated soils should contain a much larger quantity of toxic volatiles and gel-like root excretions (Fig. 4) and fewer microorganisms than cultivated ones, especially soils under bare fallow and intertilled crops.

The properties of microorganisms and plants discussed may be readily explained from an evolutionary point of view (Mechnikov 1947, Samtsevich and Mironova 1969). If microorganisms did not regulate their development, organic substances in the soil could not be accumulated, and roots that have no protective means would be "eaten" by microbes as soon as they appeared. Without taking into account the interrelationships between plants, soil and microbes it is impossible to understand the life of the soil and to effectively control its fertility.

SUMMARY

Comparative data obtained over three years are presented. They concern the number of microorganisms and content of organic matter in southern chernozem under closed oak stands and bare fallow on the steppe. In bare

fallow soil, in spite of the considerably smaller amount of organic matter, the number of microorganisms is greater than in soil under forests. Root caps and suction and exomotic roots are shown to form transparent gel-like protective caps and to slough off a large number of plant cells during their growth. They can be easily seen under the microscope when stained and play a significant role in the life of plants and in soil-forming processes.

It is shown that the reason for the smaller number of microorganisms in virgin soils compared with cultivated ones is the accumulation in the former of toxic volatiles and the excretion by root tips of phytoncide substances which inhibit the development on the roots and in the rhizosphere not only of pathogenic but also of many other kinds of saprophytic microorganisms. The maximum number of microorganisms in the soil was found during the winter and spring period.

The author considers that the role of mechanical working of the soil consists not so much in supplying it with atmospheric oxygen as in the removal from it of toxic volatile substances.

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EFFECT OF INCREASING AMOUNTS OF NITROGEN ON THE MICROBIAL TRANSFORMATION OF STRAW IN SOIL

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Direct straw application to the soil has assumed greater importance as a manurial treatment in our country. The addition of inorganic nitrogen is required in most cases (Ansorge 1966, 1967, Kick 1965, Wagner 1964 and many others). Phosphorus and sulphur addition could be certain advantage in some cases (Stewart et al. 1966).

So far, problems on the relationship between the straw and nitrogen effects have not yet been solved. This paper deals with the effect of nitrogen on the mineralization rate and stabilization degree of organic matter of applied straw.

METHODS AND MATERIALS

The following model experiments were carried out: soil was taken from the arable horizon of slightly leached black soil at Ruzyně. It was sieved by a 2 mm sieve. 500 g of this sieved soil were put in 1000 ml Erlenmeyer flasks. Continuous flow of CO_2-free air was allowed to pass through the individual flasks in an approximate amount of 2 litres in an hour. Carbon dioxide evolved in soils was absorbed in NaOH, and the amount of carbon-

Table 1
Characteristics of the soil and straw used in experiments

	Soil	Straw
C_{ox} (% in oven dry matter)	1.48	36.4
N_t (% in dry matter)	0.143	0.47
NH_4^+—N (mg % of dry matter)	11.8	—
NO_3^-—N (mg % of dry matter)	1.3	—
pH (in H_2O)	6.9	—
pH (in KCl)	6.8	—
Respiration activity:		
(mg CO_2/100 g/hour) B (basal)	0.64	—
NG (amended)	27.6	—
Microbe counts: (in 1 g of dry matter)		
No. of Bacteria (meat agar)	6.7×10^7	3.7×10^5
No. of Bacteria (starch agar)	2.1×10^7	6.2×10^6
No. of Actinomycetes (starch agar)	9.4×10^5	5.8×10^6
No. of Fungi (Jensen agar)	3.4×10^4	2×10^5

ates was determined titrimetrically after the addition of $BaCl_2$. The flasks were incubated at 28 °C for 99 days.

Soil was moistened by distilled water or by $(NH_4)_2SO_4$ solution to adjust the nitrogen content according to the experimental scheme. Half of the flasks with soil were enriched by ground wheat straw in an amount of 1% C. Straw was previously moistened by distilled water to about 50% by weight. The moisture content of the flasks was readjusted every week. The characteristics of the used soil and straw are given in Table 1.

RESULTS AND DISCUSSION

The mean daily C-mineralization rate of individual variants is given in Table 2.

Table 2

Mean daily mineralization (mg% C)

Incubation time, days	Without straw				Straw added (1% C)			
	Nitrogen addition				Nitrogen addition			
	0	10 mg %	30 mg%	100 mg%	0	10 mg %	30 mg %	100 mg %
2	1.82	2.16	2.21	2.18	2.23	2.54	2.68	3.07
5	1.11	1.48	1.35	1.32	1.64	1.35	2.73	3.64
8	0.66	0.98	0.87	0.90	1.25	2.38	4.28	4.12
11	0.42	0.53	0.68	0.61	1.86	3.65	4.96	4.64
15	0.40	0.42	0.54	0.56	2.18	3.91	4.84	4.95
22	0.38	0.39	0.51	0.53	3.25	4.18	4.85	4.55
29	0.39	0.36	0.43	0.35	3.08	4.28	4.02	4.08
36	0.36	0.34	0.38	0.37	2.82	3.31	3.16	3.45
43	0.36	0.31	0.35	0.31	2.64	2.96	2.21	2.68
50	0.35	0.30	0.30	0.27	2.36	2.64	1.93	1.99
57	0.33	0.29	0.31	0.22	2.11	2.16	1.71	1.62
64	0.35	0.30	0.28	0.22	1.96	2.03	1.23	1.28
71	0.34	0.28	0.24	0.21	1.92	2.02	1.11	0.83
78	0.32	0.28	0.22	0.21	1.85	2.10	0.89	0.69
85	0.32	0.27	0.22	0.22	1.88	2.04	0.72	0.64
92	0.33	0.29	0.22	0.21	1.67	1.93	0.63	0.70
99	0.33	0.27	0.22	0.20	1.73	1.96	0.74	0.59

The daily respiration rate of the control soil decreases to about 15% of the initial value during the whole experiment. The most pronounced decrease of respiration intensity occurs in the first week of incubation.

Owing to the addition of nitrogen to the soil, the respiration intensity rises to about 120% in the first and second day of the experiment (Fig. 1). But it decreases gradually during the incubation time even more rapidly than in the control soil. The ratio of respiration rates of individual soil samples amended with N is shown in Fig. 2.

The total amount of mineralized C during the whole incubation time is 40.73 mg% in the control soil, 39.73 mg% in the soil samples with the addition of 10 mg% N, 41.04 mg% in those with 30 mg% N, and 38.30 mg% in samples amended with 100 mg% N.

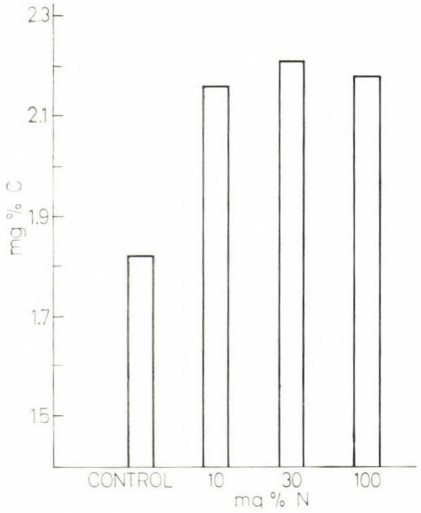


Fig. 1. Effect of added N on the soil respiration at the beginning of the incubation period

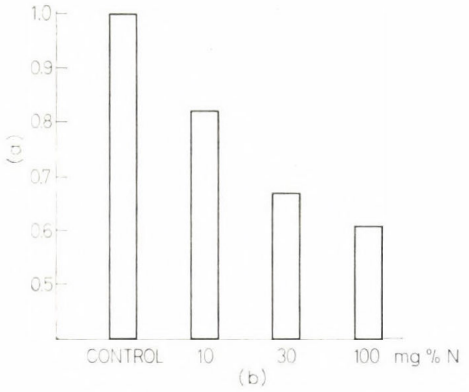


Fig. 2. Ratio of respiration rate of control (a) N-amended soil, (b) at the end of the incubation period

Fig. 3. Effect of added nitrogen on the respiration of soil amended with straw at the beginning of the incubation period

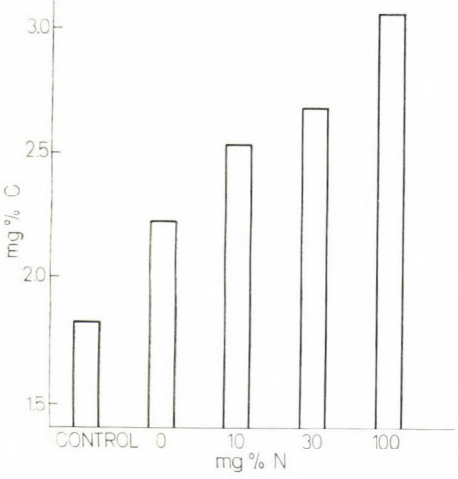
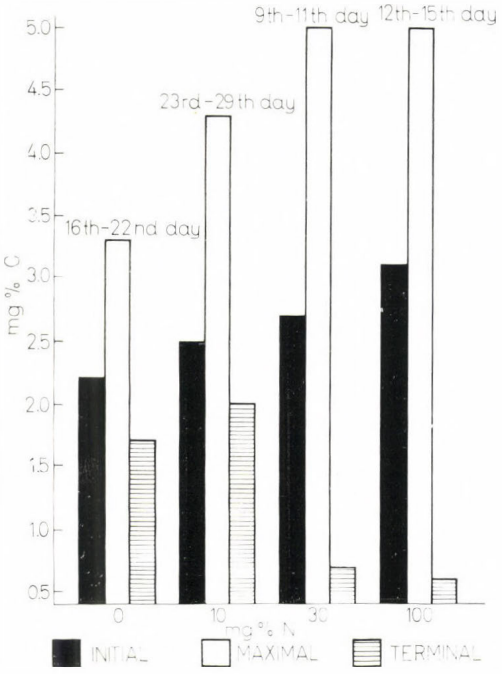


Fig. 4. Initial, maximal and terminal respiration rate of the straw variants



The effect of added straw on the respiration rate at the start of the experiment is of the same range as the effect of the added nitrogen (Fig. 3). In contrast to the N-effect, the added straw allows the rising of respiration rate of soil for some weeks (Fig. 4). The maximum respiration rate reached after the straw addition depends on the nitrogen addition, i.e. on the C/N ratio.

After reaching its maximum respiration intensity, the respiration rate of the soil-straw-mixtures decreases. The decline of the respiration curve is more pronounced after the greater doses of applied N (Fig. 4). The proportion of the respiration rate of straw variants at the end of the experiment is also given in Fig. 4.

Neglecting the priming effect of added straw, we can compute the degree of mineralization of added straw to 17.76%, 22.44%, 18.20% and 18.65% without and after an addition of 10, 30 and 100 mg % N respectively (Fig. 5).

The effect of added straw on the soil respiration expressed by the ratio of respiration of soil sample with and without straw under the same conditions of added N changes during the incubation. Its maximum coincides temporally with the maximum respiration rate of the straw-amended variants or it takes place a little later (Fig. 6).

The ratio of the maximum value of the straw-effect in the course of experimental incubation and the same value at the and of incubation could be used as a test of stabilization of organic matter of added straw. Fig. 7

Fig. 5. Total mineralization of both soil and straw organic matter

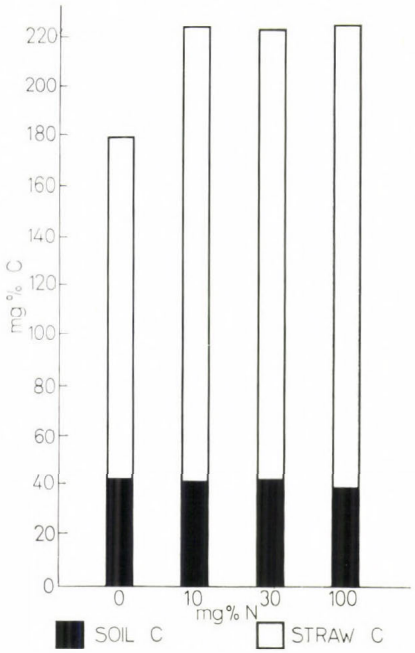
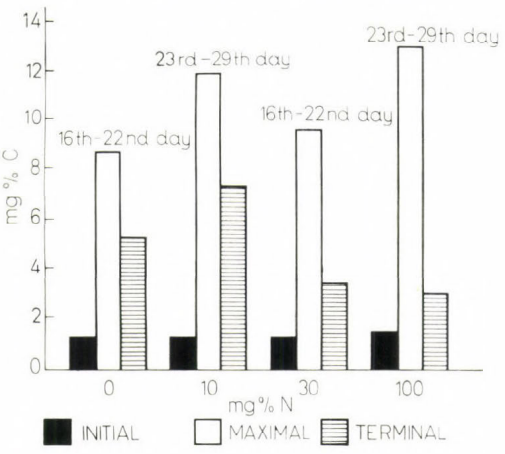


Fig. 6. Ratio of respiration rate of straw (a) unamended, (b) variants



shows that the least dose of applied N does not influence the stabilization of the substrate. That is perhaps why the same dose causes the extended mineralization of straw compared with the variant without added N. The medium and the greatest doses stabilize the organic matter of straw.

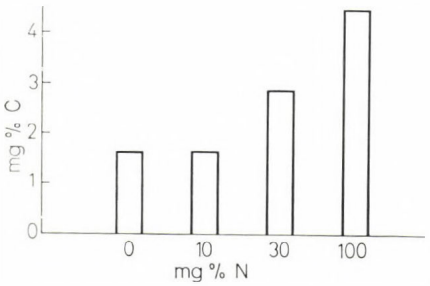


Fig. 7. Ratio of (a) the maximal and (b) terminal effect of added straw (test for stabilization degree)

SUMMARY

We carried out model experiments to study this problem. Wheat straw in adequate quantity (equal to 1% of C referred to the mixture) was added to samples of a soil taken from the arable layer, furthermore 0, 10, 30 and 100 mg% of nitrogen in the form of ammonium sulphate. Samples were incubated for 99 days at 28 °C. CO_2 production was determined from time to time during the whole period of incubation.

Soil respiration rises immediately after the addition of both straw and nitrogen. While the respiration rate decreases with time in the variant soil + nitrogen, it rises first in the variant soil + straw and later declines again. The amount of the mineralized soil carbon does not change much during the incubation period if nitrogen was added (in an amount of about 40 mg %) The added straw was mineralized to an extent of 17.76, 22.44, 18.20 and 18.65% respectively in the control and the variants with increasing amounts of nitrogen added.

The maximum stabilization of the organic matter originating from the straw added was reached with the addition of 100 mg % of nitrogen. We did not obtain any stabilizing effect in the variant with less quantity of nitrogen added.

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DECOMPOSITION OF THE OUTWARD WAXY FILM OF WHEAT STRAW BY SOIL MICROORGANISMS*

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The microbial decomposition of the main constituents of wheat straw when it is buried in the soil for agricultural purposes, is well known. But the microbial attack of exiguous constituents of straw, such as the waxy film which covers the culms, has been very little investigated; so that a specific research may be interesting.

However, wax, because of its waterproof property, has an indirect influence on the microbial colonization of straw both before and after the burial. Furthermore, as the waxy matter may have a direct trophic action, researches on the microbial growth on the waxy matter and subsequently on the behaviour of microorganisms when they are grown in the presence of waxy matter have been carried out.

MATERIAL AND METHODS

The waxy matter is extracted from the straw by Soxhlet apparatus with petroleum ether.

The growth of microorganisms on waxy matter is ascertained by the three following methods:

a) The wax is previously either directly hot-distributed on the surface of plates or also hot-arranged previously upon a film of matter not suitable to support the microbial growth: i.e. highly substitued cellophane. The medium in the plates lacks carbon sources and contains the following mineral salts: K_2HPO_4 2.00 g; $MgSO_4$ 0.50 g; NaCl 0.10 g; $CaCl_2$ 0.50 g; $FeSO_4$ traces; Bacto agar (Noble, Difco) 30 g; distilled water 1000 ml. One of the following forms of nitrogen is present: $(NH_4)_2SO_4$, $NaNO_3$, urea and calcium cyanamide (technical grade). Nitrogen concentration in the medium is the same of 2.00 g/l of $(NH_4)_2SO_4$. The wax is inoculated with a water-suspension (0.500 g/l) of twelve different Sardinia soils or with the following fungi: *Alternaria crassa* (Sacc.) Rands, *Alt. geophila* Doszewska, *Alt. dauci* (Kühn) Gr. et Sk. f. sp. *solani* (Ell. et Mart.) Neegr., *Alt. tenuis* Nees, *Chaetomium cochliodes* Palliser, *Fusarium* Link sp. 1, *Fusarium* Link sp. 2, *Helminthosporium sativum* Pammel et al., *Humicola grisea* Traaen. After incubation for 7 days at 26 °C the growth is valued by visual and microscopic surveys.

* The work was supported by Consiglio Nazionale delle Recerche, *Centro di Studio per la Microbiologia del Suolo.*

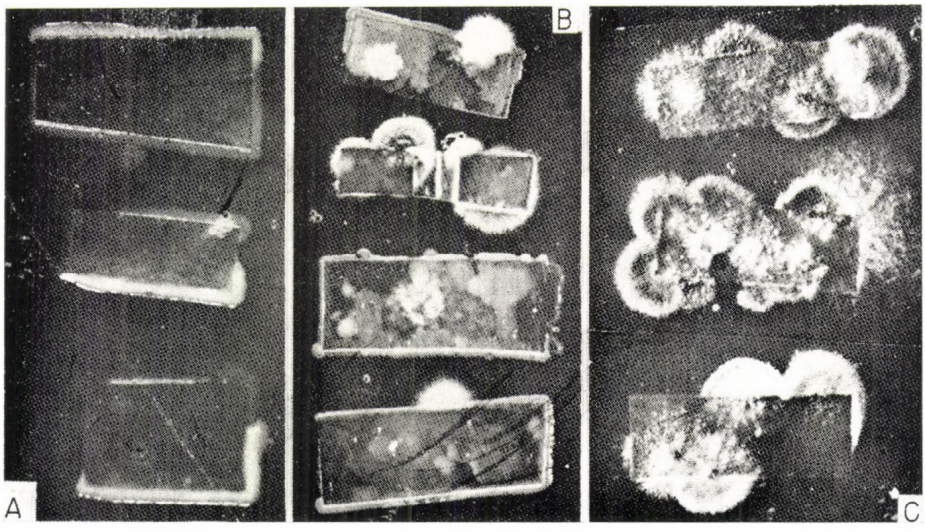
b) 50 g of siliceous sand, carefully deprived of organic matter, are placed in 100 ml Erlenmeyer flasks and mixed with: 1) 2 g of minced wheat straw; 2) 2 g of minced wheat straw deprived of wax; 3) 1.7 g of minced wheat straw supplemented with 0.3 g of waxy matter. The mixtures are sterilized and then moistened with 40 ml of sterile Čzapek-Dox solution deprived of saccharose. The above listed fungi are inoculated and then incubated for 30—60—90 days at 26 °C. The growth is evaluated by determining nucleic acids with the method of Crestfield et al. (1955). Absorbance was measured at 260 mμ on Unicam SP800B recording spectrophotometer.

c) Glass balls of about 1.5—2 mm in diameter were covered with melted wax. The balls were in the following medium: distilled water 1000 ml, $Ca(H_2PO_4)_2$ 0.5 g; K_2HPO_4 0.75 g; $MgSO_4 \cdot 7H_2O$ 0.25 g; and also containing, as nitrogen source $(NH_4)_2SO_4$ 0.75 g or, in parallel tests, equivalent quantities with respect to nitrogen, of urea and calcium cyanamide; pH was adjusted to 6.8. Every tube was inoculated with a drop of suspension of 0.5 g of nine different soils in 1000 ml of water. After incubation at 26 °C, nephelometric estimation of the growth was made.

RESULTS

As far as the first method is concerned, the results allow us to point out the following. On wax films inoculated with soils, the microbial growth was frequently positive, according to the nature both of the soil and of nitrogen source. In this regard, urea and calcium cyanamide have given better results: in fact, after twenty days of incubation, the average number of fertile dowels was 91% for urea, 83% for calcium cyanamide and 75% both for ammonium sulphate and for sodium nitrate. Furthermore, dif-

Fig. 1. Examples of microbial growth on the waxy layer inoculated with soils: A — in the presence of urea the bacterial growth is prevalent; B — in the presence of calcium cyanamide the growth of microfungi is associated with the bacterial growth; C — in the presence of ammonium sulphate, microfungi prevail



ferent nitrogenous sources seemed to qualitatively influence growth too (Fig. 1). Still considering now the tests carried out with fungi, we have seen that growth has always been positive; for some fungi, like *Chaetomium cochliodes* and *Fusarium* sp, the growth has been particularly abundant.

The results obtained with the second method (Table 1) show that the straw wax influences the growth of the microfungi in various ways. If we

Table 1

Native nucleic acids present in the single cultures

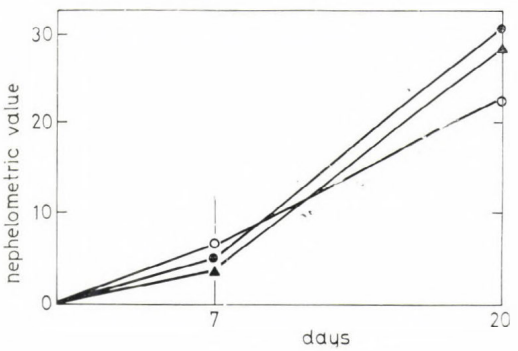
Microfungi	Nucleic acids in mg × 10^2		
	A	B	C
Alternaria crassa	816	1.208	1.416
Alt. gaophila	1.384	1.580	1.912
Alt. dauci solani	1.024	1.040	1.568
Alt. tenuis	952	1.264	1.680
Chaetomium cochliodes	1.472	1.704	2.448
Fusarium sp. 1	1.224	1.384	1.448
Fusarium sp. 2	1.016	1.336	1.576
Helminthosporium sativum	1.080	1.560	1.984
Humicola grisea	1.296	1.480	2.424
Average values	1.128	1.392	1.736

Note: A — minced straw: B — straw minced and deprived of its waxy material; C — straw-like in B, but to which an excess quantity of waxy material has been added again.

compare the growth of fungi in the presence of straw, with the growth in the presence of straw deprived of its wax, it is plain that the straw tissues free of wax allow a growth slightly greater than that which we obtain from native straw. However, if we compare the growth of fungi in the presence of straw without wax, but to which free wax had been added, we see that in the medium containing wax, growth has been greater in every case. This indicates that wax added to the other constituents favours their utilization.

The results obtained with the third method have given further evidence for the microbial utilization of the wax. In fact nephelometric determination carried out at the seventh and at the twentieth day showed a notable growth, depending on the soil. Moreover we observed that the nitrogen source influences both the intensity and the dynamics of the growth. Indeed whilst in the first few days growth was

Fig. 2. Average values of the microbial growth in cultures on liquid medium inoculated with various soils and in the presence of various sources of nitrogen. ○ ammonium sulphate, ● urea, ▲ calcium cyanamide



greater in the presence of ammonium sulphate, later on, it turned out to be clearly greater in the presence of urea and of calcium cyanamide (Fig. 2). This result agrees with the one of the first method and shows that an appreciable growth of soil microorganisms occurs in the presence of wax as a carbon source.

The researches concerned in this paper show that the waxy material present on the wheat straw is utilized by soil microorganisms and may support their growth. Such utilization seems to be influenced by the nature of the source of nitrogen present in the medium. Furthermore, the waxy material of the straw has an influence in some way on the decomposition of the other constituents of straw when it is buried in the soil.

SUMMARY

Researches on the microbial decomposition of the outer waxy layer of wheat straw have been carried out, to investigate the decomposition of straw buried in the soil for agricultural purposes. The results show that:

1. the waxy material extracted from wheat straw certainly supports microbial growth;
2. the presence of wax stimulates microbial growth supported by other straw constituents;
3. microbial growth concerned in the decomposition of wax is influenced both at a quantitative and at a qualitative level by the form of nitrogen present. Urea and calcium cyanamide turns out to be particularly effective, as far as bacterial growth is concerned, even if fungi are not damaged.

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MICROBIAL PROCESSES RELATED TO THE DECOMPOSITION OF ORGANIC MATERIAL WITH VARIOUS NITROGEN CONTENTS

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Model experiments under controlled temperature and moisture conditions were conducted so as to throw light on microbiological processes related to mineralization of organic residues. For this purpose soil samples were taken from chernozem-smolnitze brown forest soil, grey forest soil and chernozem to which triturated alfalfa hay, peat and straw were added, and composted over 6 months in variants with and without nitrogen and phosphorus.

MATERIAL AND METHODS

This study aimed to assess changes in microflora particularly in microbiological processes related to the nitrogen regulation in soil as affected by the organic substances of different nitrogen content. Studies still have to be done to control mineralization of organic residues and clarify the nitrogen regulation in the soil. Microbiological and biochemical tests as well as the counting of the number of microorganisms were done using conventional methods (Fedorov 1951). Hydrolyzable nitrogen, total nitrogen, fulvic and humic acids were also determined.

RESULTS

Total and hydrolyzable nitrogen showed an increase in soil only in the alfalfa variant (from 0.123 per cent in the control to 0.139 per cent) both in fertilized and non-fertilized variants. Quantitative changes of hydrolyzable nitrogen were also assessed in the alfalfa variant, 7 mg/kg soil, in the untreated variant and 8 mg with nitrogen and phosphorus fertilizing. Changes of the fulvic and humic acid contents were noted at the end of the experiment. The quantity of the nitrogen of fulvic acids was higher by 0.5 g than the control in the treatments containing peat and straw and by 2.1 g higher in the treatment containing alfalfa hay calculated per 1 kg soil. Humic acids increased only slightly but to a higher degree in the variant with alfalfa, to 0.47 g/kg soil. NP fertilization slightly increased the nitrogen content of both fulvic and humic acids.

Changes of microbiological processes were followed to clarify changes occurring in the nitrogen regulation of the soil. Almost all data obtained point to a higher effect of the kind of organic substance rather than of the soil type on the related processes. Consequently, data only

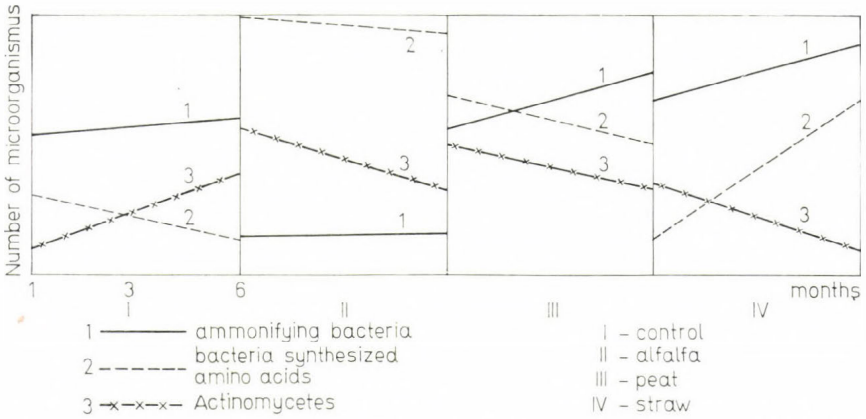


Fig. 1. Changes in the amount of the soil microflora. I = ammonifying bacteria, 2 = bacteria synthesizing amino acids, 3 = Actinomycetes. I=control, II = alfalfa, III = peat, IV = straw

of few soil types are reported to illustrate the foregoing. Substantial changes occurred in the composition and not in the number of soil microflora (Fig. 1). Hence, following addition of alfalfa hay multiplication of bacteria was noted, which, during their development used mineral nitrogen and participated in synthesis of protein nitrogen in soil. The number of ammonifying bacteria was reduced and that of actinomycetes increased in this variant. There were changes in peat and straw variants similar to those of the control soil which was under permanent temperature and moisture conditions for 6 months. The tendency of ammonifying bacteria to increase in number could be observed in both variants, especially on the effect of a longer incubation period. In the treatments with straw, the number of the bacteria utilizing the mineral nitrogen also increased.

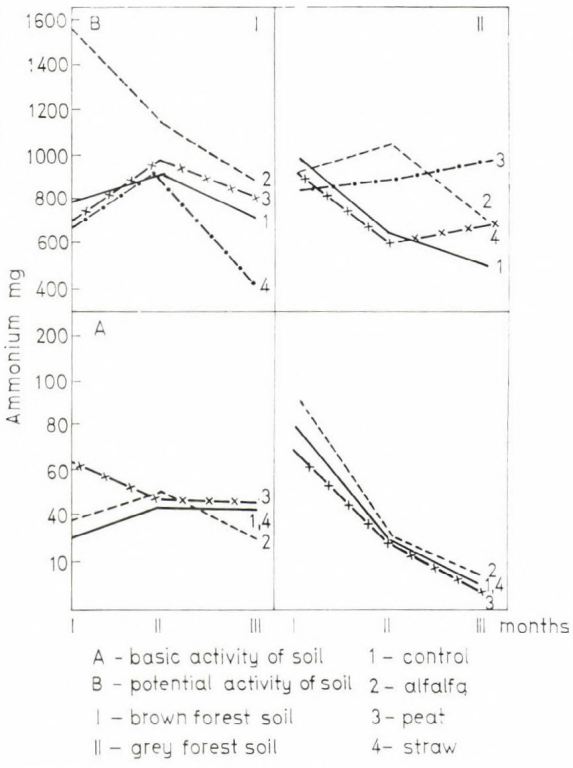
Bearing in mind physiological characteristics and participation in mineralization processes of the different groups of microorganisms mentioned (Voinova 1963), the following inferences may be drawn:

a) Application of alfalfa hay; an organic substance of higher nitrogen content, enhances ammonification intensity and accumulation of available nitrogen in the soil, which is a prerequisite for the multiplication of bacteria, using mineral nitrogen. The latter ones store nitrogen in the soil in the form of organic substances and are the basis of amino acid accumulation, fulvic and humic acid, etc. Therefore, available nitrogen obtained during mineralization of the organic residues of high nitrogen level is not subjected to washing off and storage in the soil.

b) In variants having low nitrogen containing peat straw, a smaller amount of mineral nitrogen was liberated in the course of ammonification and in consequence even fewer bacteria occurred which incorporated the mineral nitrogen into their cells. The tendencies noted do not show basic differences in variants with and without fertilizers.

Different experimental variants did not show changes in the number of microorganisms connected with the breakdown of organic phosphorus compounds in the soil.

Fig. 2. Activity of ammonification of the soil. 1 = control, 2=alfalfa, 3=peat, 4=straw, A = basic activity of soil, B = potential activity of soil, I = brown forest soil, II = grey forest soil



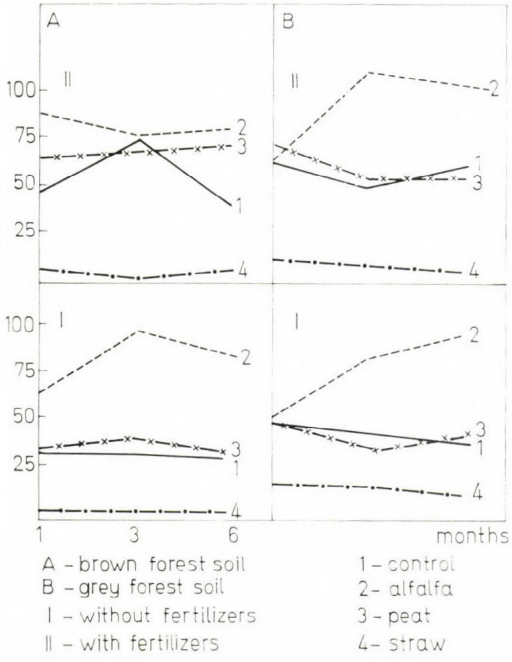
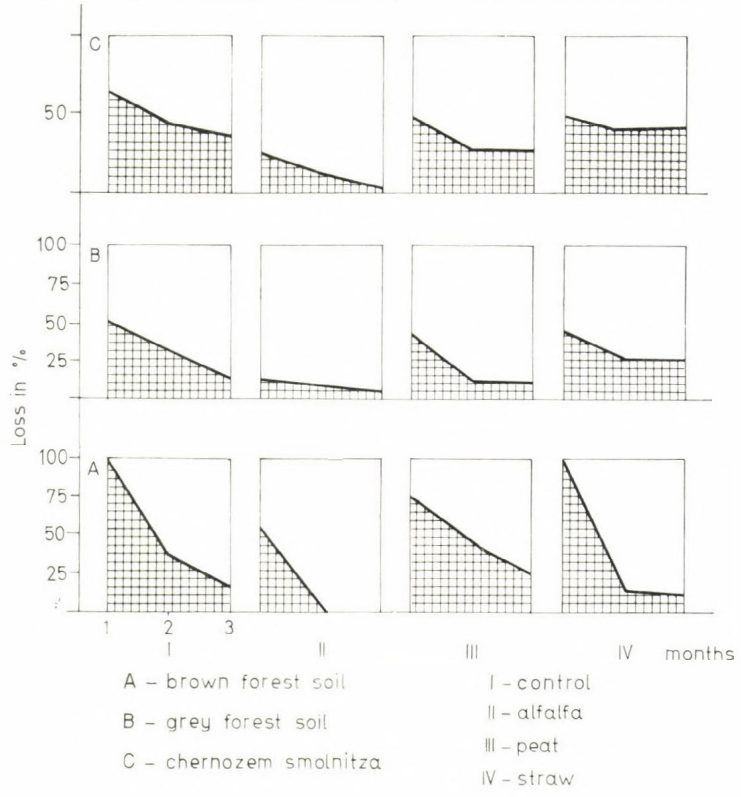


Fig. 3. Activity of nitrification of the soil. A = brown forest soil, B = grey forest soil, I = without fertilizers, II = with fertilizers, 1 = control, 2 = alfalfa, 3 = peat, 4 = straw

To throw light on changes of the nitrogen regulation in soil at mineralization of organic substances having different nitrogen levels, basic ammonification and nitrification were studied along with potential ammonification and nitrification (Voinova 1963, Peive 1961). As compared to the control, there were no important changes in intensity of basic ammonification in single variants. Tendencies towards increased intensity of this process with potential ammonification in the alfalfa hay variant were noted (Fig. 2). Essentially these changes are similar to those noted in the content of ammonifying bacteria, i.e. ammonification proceeds more rapidly in the alfalfa hay variant.

Assessment of nitrifying activity (Fig. 3) shows particularly abrupt changes as induced by different plant materials. Hence nitrification activity of soil is greatly enhanced in the alfalfa hay variant and quite similar to the control in the latter variant while a rapid decrease of the nitrification activity is noted in the straw variants. In non-fertilized variants tendencies are similar to those for variants treated with nitrogen and phosphorus. Nitrogen fertilization in a dose of 60 kg/ha did not cause decrease in the negative effect of straw on nitrification processes in the soil. Our concept

Fig. 4. Cellulose-decomposing activity of the soil. I = control, II = alfalfa, III = peat, IV = straw, A = brown forest soil, B = grey forest soil, C = chernozem-smolnitza



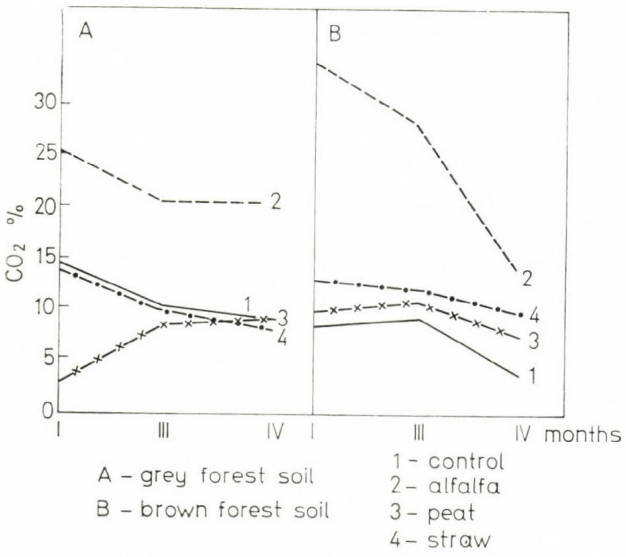


Fig. 5. Changes of the CO_2-content of the soil air. A = grey forest soil, B = brown forest soil, 1 = control, 2 = alfalfa

is that a more prolonged mineralization period of straw probably induces the uptake of soluble organic substances which as we know (Nikitin 1960) inhibit nitrification. Data on the cellulose-decomposing activity (Fig. 4) confirm the suggestion that straw mineralization is less intensive compared to that of alfalfa hay. The filter paper put in the variant with alfalfa hay was completely decomposed as early as at the end of the first month (Fedorov 1961) and at the same time a certain portion remained unchanged in the peat and straw variants up to the end of the third month.

The CO_2 production of some experimental variants differs from each other markedly. The CO_2 production in the treatment with alfalfa hay was initially very intensive then it gradually increased; in the treatments containing peat and straw the change was substantially less, though the total amount of CO_2 was much less (Fig. 5). A similar tendency was noted in the fertilized variants.

DISCUSSION AND CONCLUSION

The experiments mentioned give a good explanation to understanding why we have relatively high N-quantities in total nitrogen, hydrolizable nitrogen, as well as in the quantities of fulvic- and humic acids in the course of the mineralization of the high nitrogen containing plant residues.

1. In the case of higher nitrogen level in plant residues, microbiological processes correlated with the elimination of mineral nitrogen in the soil are intensified and accelerated during mineralization. These processes however, determine the development of microorganisms, the cells of which

take up and retain the mineral fertilizer thus storing it in the soil. Decreasing organic substances under the conditions of such an intense mineralization greatly stimulates nitrification processes and accumulation of microbes in soil. Consequently conditions are created for enriching soil with nitrogen, fulvic and humic acids.

2. Plant residues of low nitrogen level are mineralized less rapidly and gradually induce decrease in the nitrification processes and rather slow elimination of available nitrogen from organic soil substance.

3. Addition of inorganic nitrogen in the dose up to 60 kg/ha when applying organic substances of low nitrogen level basically enhances mineralization of cellulose and only slightly affects ammonification, which is related to the supply of the energy requirements for nitrification. Consequently it is not possible to expect microbiological processes to be related to accumulation of nitrogen in soil. Studies on higher nitrogen doses (forms and techniques applied) should be conducted with a view to controlling microbiological processes for enrichment of soil with nitrogen and humic acids.

4. Nitrogen content in plant residues to a great extent determines the course of mineralization processes, obtained from intermediate substances and humification of soil.

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INTERACTION BETWEEN ORGANIC COMPOUNDS AND INORGANIC NITROGEN IN SOIL

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The role of organic compounds in transformation of inorganic nitrogen in soil, particularly in oxidation process of ammonia has not yet been sufficiently established. According to Winogradsky (1953) organic substances of soil are specific inhibitors of nitrification, however, other authors (Jensen 1950, Pandalai 1936, Stevens and Withers 1910) have a different opinion in that respect.

The aim of this paper was to explain the influence of processes connected with microbial decomposition of organic compounds on the conversisuo of inorganic nitrogen in the soil.

MATERIAL AND METHODS

The transformation of inorganic nitrogen was investigated: *a)* in a sandy soil incubated in jars at 27 °C, *b)* with the same soil by means of percolation technique. 2 g of ammonium sulphate or potassium nitrate, as the nitrogen source were added to 1 kg of soil. In experiments with percolators the 0.01 M solution of these nitrogen compounds — in amount 320 ml per 50 g of soil, was used. The following organic compounds were added to the soil: glucose, starch, casein hydrolyzate and gelatin.

In order to bring about the proliferation of nitrifiers, soil with ammonium sulphate was incubated in jars or percolated 14 days before addition of organic compounds. Then the samples of soil from jars were analyzed after 21 and 90 days of incubation. The experiments using percolators lasted 15 days. Samples of percolating solution were analyzed every 3 days during the experimental period. At the end of the experiments the samples of percolated soils were also analyzed.

The number of proteolytic bacteria, ammonifiers, bacteria assimilating nitrates and bacterial groups reducing them to nitrites was determined. Selective nutrient media for culturing the tested bacterial groups were applied for this purpose.

Chemical analyses included the determination of amounts of ammonium-, nitrite- and nitrate nitrogen (by colorimetric methods). Moreover, organic nitrogen was determined by the Kjeldahl method after extraction of inorganic compounds.

The pH of soil samples was measured as well.

RESULTS

Experiments with soil incubated in jars. Figure 1 shows the results of the experiment on the influence of carbohydrates on transformation of ammonium nitrogen in the soil.

The carbohydrates added to the soil, especially glucose, caused a vigorous proliferation of proteolytic bacteria, bacteria assimilating nitrates and bacteria reducing them to nitrites. Metabolites of carbohydrates being formed by mentioned and other groups of microflora did not inhibit the oxidation of ammonium nitrogen, but even influenced the increase of this process after 21 days. At this time the amount of nitrates was greater in the soil with added carbohydrates compared to the control. The amount of this form of nitrogen decreased however, after 90 days. In this period, considering that ammonium nitrogen was exhausted, the content of nitrates decreased due to their conversion to organic nitrogen by microorganisms (Fig. 1).

The addition of organic nitrogen compounds to soil involved, similarly as in the case of carbohydrates, a marked increase in number of the tested bacterial groups (Fig. 2). After 21 days it was more intense in the presence of casein hydrolyzate than in that of gelatin. At this period vigorous disturbances in nitrification process were noted in soil enriched with organic nitrogen compounds. The second step in nitrification was inhibited more strongly than the first, affecting the accumulation of nitrites in the soil. After 3 months no disturbances in nitrification were noted in soil with organic nitrogen compounds. At this time the amounts of nitrates were even higher in the presence of casein hydrolysate compared to the control. This was the result of nitrification of some amount of ammonium nitrogen derived from the breakdown of this compound.

Figure 3 presents the results of the experiment on the effect of organic compounds on transformation of nitrate nitrogen added to soil in the form of potassium nitrate.

The addition of glucose or casein hydrolyzate to soil with potassium nitrate caused the vigorous proliferation of the tested bacterial groups, similarly as in the case of experiments with ammonium sulphate. This was observed even in the later experimental period.

After 21 days the decrease of nitrate nitrogen in soil with organic compounds was noted. However, after 3 months the amount of nitrates in soil enriched with glucose or casein hydrolyzate was about 30% higher than that in the control (Fig. 3).

Experiments by means of percolating technique. It seems that the conditions under which percolation was running in our experiments were not favourable for nitrification process. Technical limitation did not allow exact regulation of speed of solution flow, thereby excessively moistening the soil. In this connection the influence of organic compounds on transformation of inorganic nitrogen in percolated soil differed to some extent from that in soil incubated in jars.

In 2 weeks' experiments, carried out by means of percolating techniques, glucose inhibited the first step in nitrification (Fig. 4) and gelatin appeared to be a strong inhibitor of the second step in this process (Fig. 5).

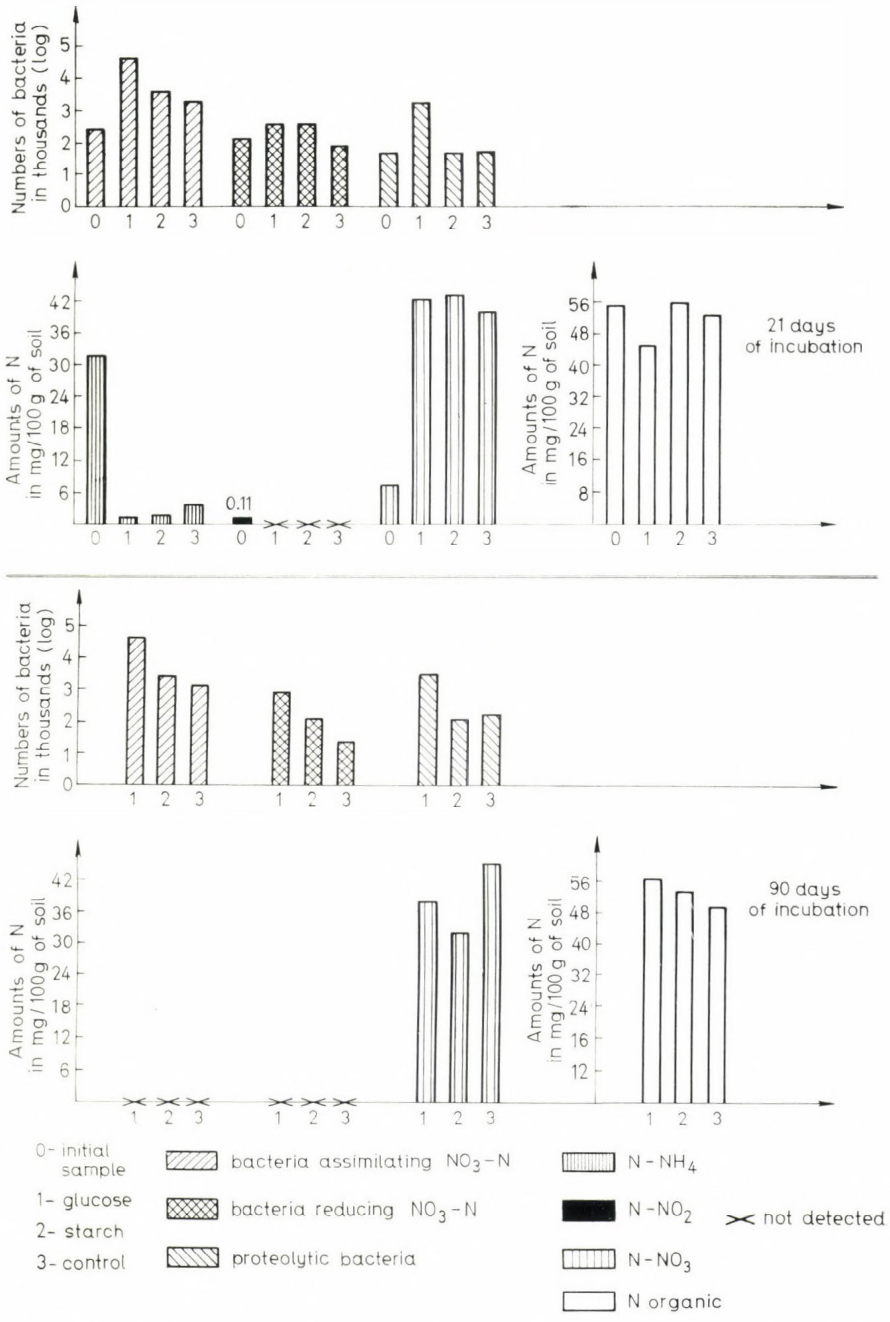

Fig. 1. Effect of carbohydrates on the development of some bacterial groups and on the transformation of $NH_4^+ - N$ in the soil

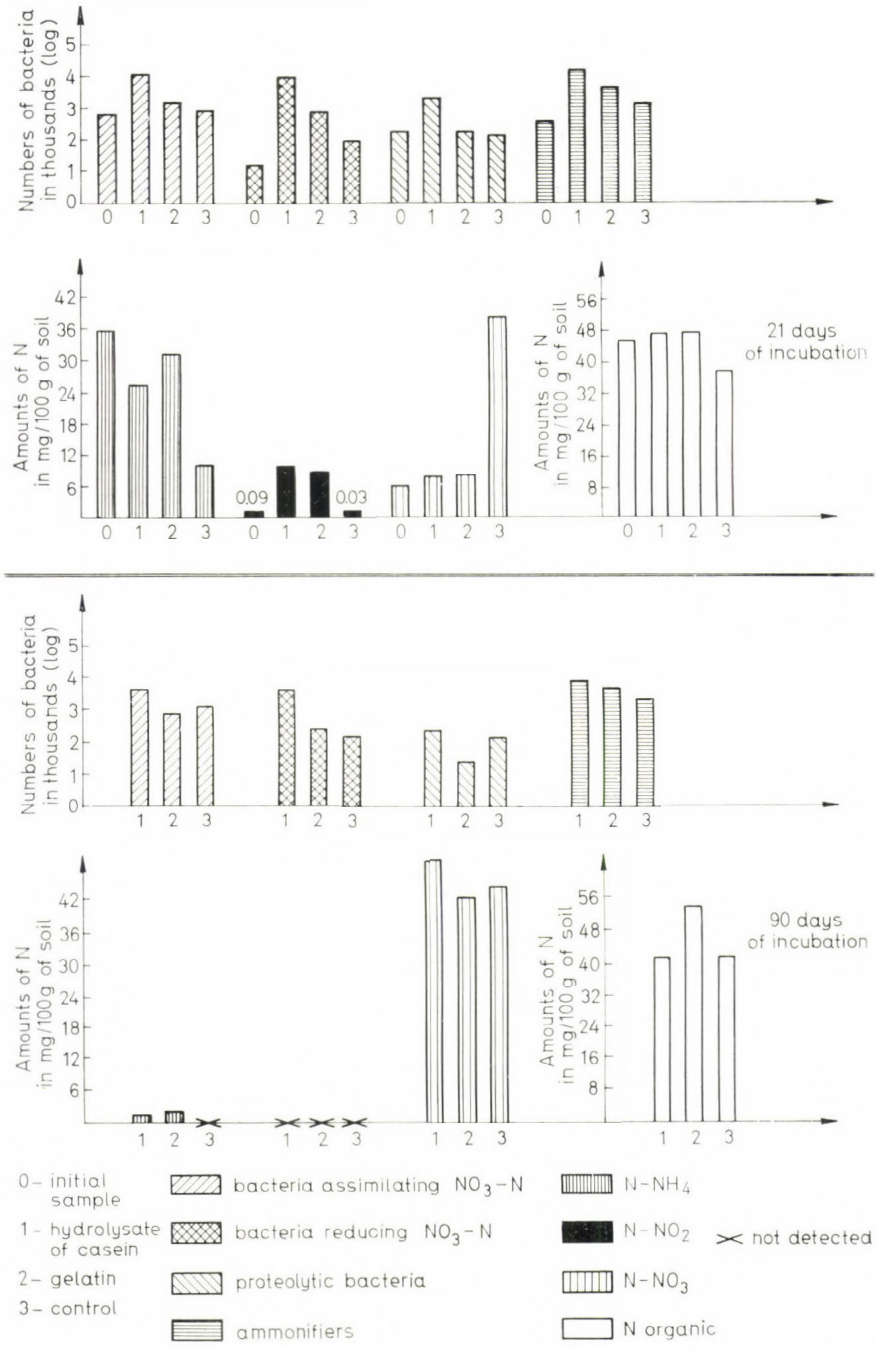

Fig. 2. Effect of organic nitrogen compounds on the development of some bacterial groups and on the transformations of $NH_4^+ - N$ in soil

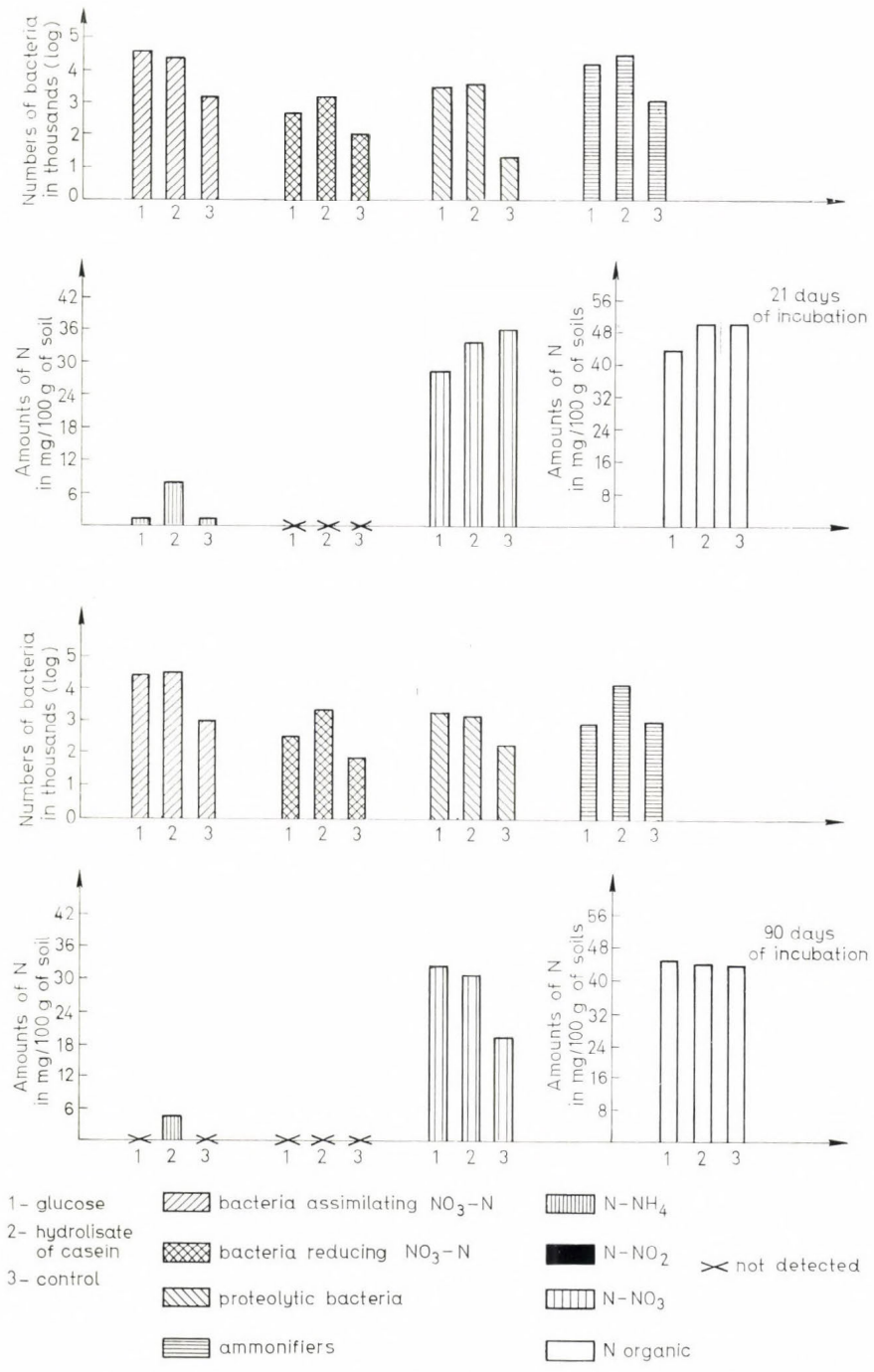

Fig. 3. Effect of organic compounds on the development of some bacterial groups and on the transformations of NO_3^- — N in soil

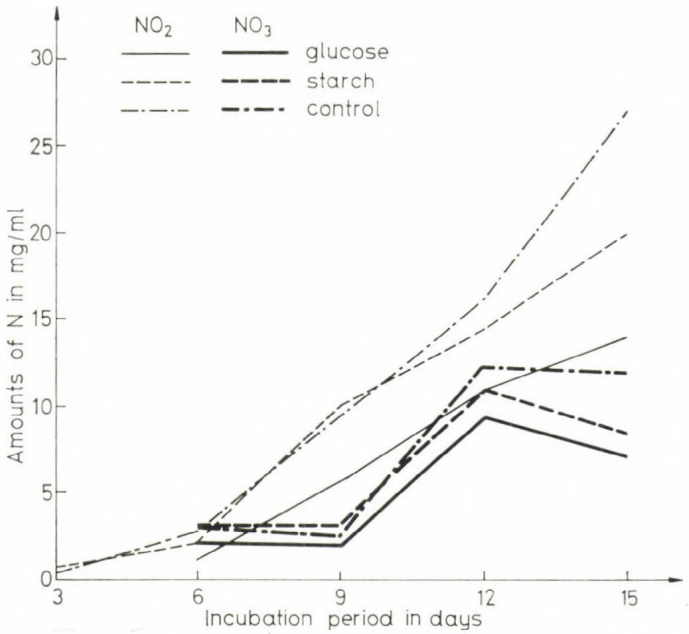

Fig. 4. Experiment with the use of percolators. Effect of carbohydrates on the oxidation of $NH_4^+ - N$ in the percolating solution

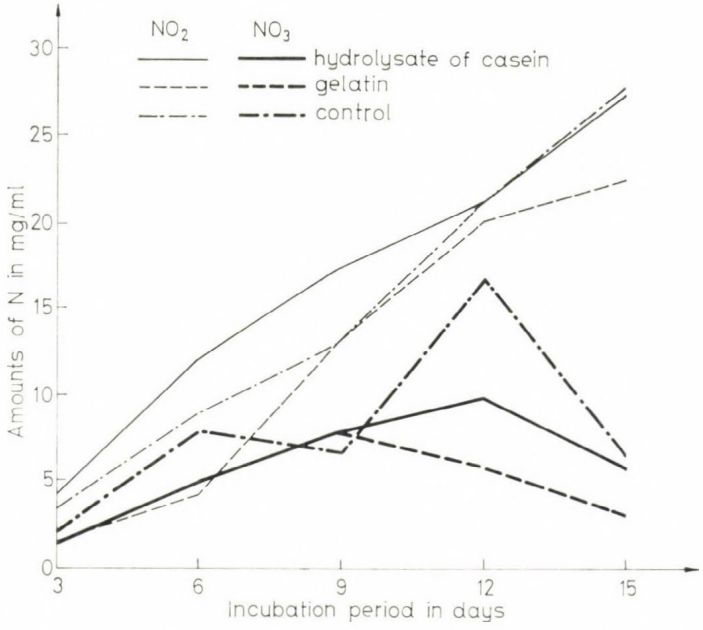

Fig. 5. Experiment with the use of percolators. Effect of organic nitrogen compounds on the oxidation of $NH_4^+ - N$ in the percolating solution

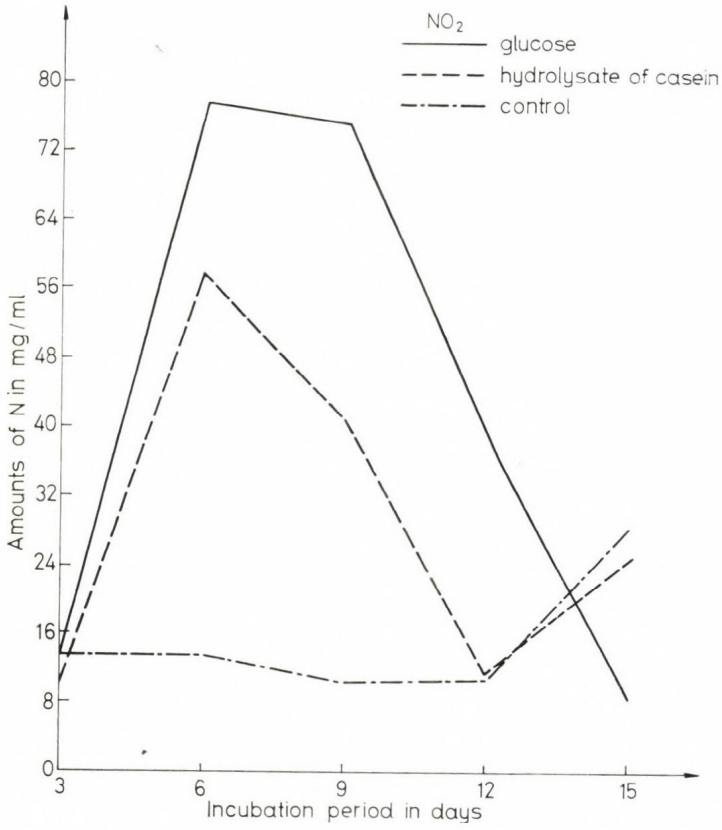

Fig. 6. Experiment with the use of percolators. Effect of organic compounds on the reduction of $NO_3^- - N$ in the percolating solution

Percolating solution contained a negligible number of bacteria, on the other hand, the percolated soil, especially in the case of treatment with glucose, was abundant in the tested bacterial groups.

There was a marked reduction of nitrate nitrogen production in percolating solution with added potassium nitrate due to the effect of organic compounds (Fig. 6). The effect of glucose on the reduction of nitrates to nitrites was stronger than that of casein.

No detectable changes of pH of incubated soil were observed in the experiments. It accounted for about 6.

DISCUSSION

In the experiments carried out the carbohydrates added to the soil did not hinder the oxidation of ammonium nitrogen. On the other hand, organic nitrogen compounds as casein hydrolysate or gelatin upset this process, particularly its second step. It occurred, however, periodically, in

the first weeks of the experiment. This finding is in agreement with the results obtained by other authors. It is possible that amino-compounds formed due to the protein breakdown might act toxically on bacteria from genus *Nitrobacter*. The ammonia liberated in excess under these conditions could inhibit the action of nitrifiers as well.

The results obtained from our investigations indicate also that the influence of organic compounds on nitrification process in soil ought to be considered on the basis of nitrogen turnover caused by heterotrophic groups of microorganisms, responsible under favourable conditions for mineralization and immobilization of this nutrient.

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MINERALIZATION AND IMMOBILIZATION OF NITROGEN WITH SOME CROP RESIDUES AS AFFECTED BY RATES OF THEIR APPLICATION, SOIL TEMPERATURE AND NITROGEN SOURCES IN A LOAM SOIL

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Crop residues are generally used as a measure to check soil drifting caused by wind erosion in Canadian prairies. The residues are left for about a year in the field and are gradually turned under the surface soil by subsequent cultural practices. Use of these residues in the field may retard depletion of organic matter but most likely will influence the soil nitrogen balance since the interaction between various factors such as temperatures, soil texture, nutrient availability, sources of applied nitrogen, pH of the soil, and microbial population status among others have marked influence on the mobilization and immobilization of nitrogen.

Extensive literature reviews on the general topic of mineralization and immobilization of nitrogen are available — Harmsen and van Schreven (1955), Jansson (1958), Bartholomew (1965) among several others. The review of McCalla and Army (1961) which pertains to the stubble mulch farming in the great plains of USA is more generally related to the present investigation. It contains practically no information on the biological aspects of the decomposition of crop residues and nitrogen transformation processes in Canadian soils. Therefore, in the first instance the laboratory investigation was undertaken to study the effects of rates of crop residues, sources of nitrogen and temperatures on the mineralization and immobilization of nitrogen applied in fertilizer form in a loam soil which was measured in terms of nitrate production.

MATERIAL AND METHODS

a) Soils: Wood mountain loam soil — a Brown group soil was collected from the south farm of the experimental station, Swift Current, Saskatchewan in May 1961. The crop residues used in this study were also grown on this soil. The soil had the following chemical and physical properties — pH 7.4; Organic matter 3.16%; total nitrogen 0.23%; Cation exchange capacity 18.4 m.e. per 100 mg of soil; nitrate nitrogen 8 ppm; water holding capacity 55.7%; moisture 10%.

b) Crop residues: Eleven crop residues — wheat, barley, oats, rye, mustard, rape, alfalfa, peas, flax, safflower and Russian wild ryegrass were used at the rate of 1 and 5 tons per acre. These residues were ground in a Wiley mill and passed through a 40 mesh sieve. They were also analyzed using A.O.A.C. procedure for moisture, total carbon, total nitrogen, lignin and cellulose and the analyses presented in Table 1.

Table 1

Results of analysis of crop residues on oven-dry basis

Crop residues	Ash %	H_2O %	Total carbon %	Total nitrogen %	C/N	Lignin %	Cellulose %
Wheat	8.28	9.9	40.04	0.32	125.0	14.5	44.5
Oats	4.22	10.2	40.23	0.66	61.0	13.4	44.6
Barley	5.83	7.9	40.65	0.49	83.0	13.4	42.4
Rye	5.45	8.3	42.64	0.64	56.6	11.6	40.8
Flax	6.60	8.9	41.70	0.58	71.9	25.3	47.2
Safflower	7.42	17.5	40.92	0.82	49.9	11.6	35.8
Mustard	3.73	11.9	36.84	0.40	92.1	18.8	49.3
Rape	7.53	9.2	41.03	0.48	85.5	24.7	48.8
Peas	4.43	15.5	40.84	0.73	56.0	12.4	32.6
Alfalfa	13.70	12.1	41.69	2.02	20.8	11.2	30.1
Grass	6.18	13.8	37.52	2.13	18.0	9.7	29.3

c) Temperatures: With higher rates of crop residues (5T/A) the temperatures of 16 °C and 27 °C were used and with the lower rate of crop residues (1T/A) it was 30 °C.

d) Nitrogen sources: NH_4OH, $NH_4/_2SO_4$ and commonly used 11—48—0 (monoammonium phosphate) were used at 0 and 100 rate of nitrogen with the exception that NH_4OH was not used with the lower rates of crop residues.

e) Incubation of soil: Eighty grams of air-dried soil on water-free basis were used in pint milk bottles in 3 replications. The soil was mixed with the ground crop residues and nitrogen was added in solution to the desired mixture level. The bottles were closed with paper caps with a 7 mm hole, weighed and maintained at the 50 % of the water holding capacity for 2, 4 and 6 weeks at 16 °C, 27 °C and 30 °C. A composite design was used for the statistical analysis.

METHOD OF ANALYSIS

Analyses for pH and NO_3^-—N are reported elsewhere by Chandra and Bollen (1960).

RESULTS AND DISCUSSION

The term "mineralization" in this paper is used in the sense that the amount of nitrogen changed from its ammonium form to the nitrate form and not in the usual sense where the organic nitrogen is changed to mineral nitrogen generally in ammonium form. The term "immobilization" is used in the same sense as described by Bartholomew (1965). Nitrate-nitrogen changes with crop residues when applied at 1T/A are shown in Table 2 and the statistical analysis for this set of experiments is given in Table 3.

Table 2

Changes in nitrate nitrogen and pH by addition of two fertilizers and crop residues (1 ton per acre)

Soil treatment	pH				NO_3^- — N, ppm				% apparent nitrification			
	$(NH_4)_2SO_4$		11—48—0		$(NH_4)_2SO_4$		11—48—0		$(NH_4)_2SO_4$		11—48—0	
	Weeks				Weeks				Weeks			
	2	4	2	4	2	4	2	4	2	4	2	4
Soil only	7.2	7.1	7.2	7.1	31	48	31	48	—	—	—	—
Soil + N	6.9	6.6	6.6	6.5	70	145	81	155	39	97	50	107
Soil + N + wheat straw	7.2	6.8	6.7	6.5	63	145	69	149	30	97	38	101
Soil + N + oats	7.1	6.6	6.7	6.7	55	125	59	133	24	77	28	85
Soil + N + barley	7.2	6.9	6.7	6.7	62	130	66	135	31	82	35	87
Soil + N + rye	7.1	7.0	6.9	6.8	60	118	65	132	29	70	34	84
Soil + N + flax	7.4	6.8	6.6	6.7	65	138	85	151	34	90	54	103
Soil + N + safflower	7.3	6.6	6.5	6.4	61	128	66	138	30	80	35	90
Soil + N + mustard	7.2	7.0	6.6	6.8	68	131	72	143	37	83	41	95
Soil + N + rape	7.3	7.0	6.7	6.8	70	121	70	131	39	73	39	83
Soil + N + peas	7.3	6.9	6.8	6.7	90	141	102	146	59	93	71	98
Soil + N + alfalfa	7.4	6.7	6.7	6.6	74	137	78	134	43	89	45	86
Soil + N + grass	7.1	6.9	6.5	6.7	86	156	97	179	55	108	64	131

Table 3

Statistical analysis for the sources of nitrogen, soil treatments and incubation time*

Soil treatments	Grass 130	Peas 120	Soil only 113	Flax *110*	Wheat *107*	Alfalfa *106*
	Mustard *104*	Barley *99*	Rape *98*	Safflower *98*	Rye *94*	Oats *93*
Sources of N	11—48—0 110			$(NH_4)_2SO_4$ 102		
Time	4 weeks 139			2 weeks 72		

* Any two means not spanned by same line are significantly different at the 5% level by the Duncan test. Any two means spanned by same line are not significantly different.

It shows that nitrate-accumulation was complete by the end of 4 weeks with applied nitrogen sources and there was about 100 per cent mineralization of the applied nitrogen in presence of crop residues. However, there was less mineralization with ammonium sulphate than 11—48—0. Although pH was somewhat lower with the later source of nitrogen, it was in the op-

timum range for the nitrifiers. The crop residues behaved differently in the mineralization of nitrogen. Rye grass showed the maximum mineralization while rye and oats had the lowest rate of mineralization as indicated by the "apparent nitrification" rate. This could be due to the decomposing nature of a plant material in a particular soil under a given set of conditions. Such trends were observed by other workers also (Bartholomew 1965). The term "apparent nitrification" is used to show the mineralization of nitrogen since there is a good possibility that some of the added nitrogen may have been immobilized in presence of a carbon source. Carbon added in form of crop residues amounts to 40 ppm in most cases and nitrogen additions ranged from 2 to 20 ppm depending upon the crop residue. However, 100 ppm addition of the nitrogen fertilizers lowered the C/N ratio to the extent that it will induce limited nitrogen assimilation and thus more nitrogen will be left for nitrification process. Amount of soluble carbon present and C/N ratio in a plant material, may also affect the processes involved in nitrogen transformation—Jensen (1929) and Winsor and Pollard (1965a). Chandra and Bollen (1960) earlier observed such differences in the nitrification process. Frederick and Broadbent (1966) have discussed

Table 4

Nitrate-nitrogen changes in soil as affected by addition of NH_4OH and crop residues

	NO_3^- — N, ppm				Increase in NO_3^- — N, over soil only, ppm				% apparent nitrification[1]			
	16 °C		27 °C		16 °C		27 °C		16 °C		27 °C	
	Weeks		Weeks		Weeks		Weeks		Weeks		Weeks	
	2	6	2	6	2	6	2	6	4	6	4	6
Soil only	25	35	45	57	—	—	—	—	—	—	—	—
Soil + N	47	88	99	126	22	54	54	69*	—	—	—	—
Soil + N + wheat straw	42	127	90	195	17	92	45	138	26	38	44	69
Soil + N + oats	41	124	78	195	16	89	33	138	25	36	49	69
Soil + N + barley	37	134	81	195	12	99	36	138	21	46	42	69
Soil + N + rye	44	128	87	209	19	93	42	152	31	40	40	83
Soil + N + flax	42	129	84	214	17	94	39	157	37	41	59	88
Soil + N + safflower	35	117	87	177	10	82	42	120	13	29	30	51
Soil + N + mustard	43	111	96	215	18	76	51	158	24	23	40	89
Soil + N + rape	41	104	93	210	16	69	48	153	17	16	35	84
Soil + N + peas	35	152	100	213	10	117	55	156	56	64	83	87
Soil + N + alfalfa	31	130	93	197	6	109	48	140	45	55	50	71
Soil + N + grass	36	176	100	208	11	141	55	151	93	88	100	82

* Probably due to denitrification.

[1] No "Apparent Nitrification" took place with crop residues at 2 weeks. Hence data for it is deleted.

in detail the "Biological interaction" involved in ammonia transformations under different environmental conditions. The results in Table 2 are in agreement with others that the crop residues applied at IT/A rate do not contribute any change to the mineralization of nitrogen under the laboratory conditions.

When higher rate of crop residues were added at 5T/A, the C/N ratio was affected and there was immobilization of nitrogen of 2 weeks and 4 weeks and there was no nitrogen left for mineralization.

Allison and Klein (1962) also observed the "tie-up" of nitrogen with different rates of additions of carbonaceous materials. The results are given in Tables 4, 5, 6 and 7. Results for 4 weeks are deleted from these tables since the trends were similar to 2 weeks' result except that there was higher nitrate-production with all the 3 nitrogen sources. Temperature markedly affected mineralization of nitrogen. Effect of temperature on nitrification is also reported earlier by other workers, Jensen (1939), Sabey et al. (1954), Frederick (1956), and Chandra and Nielsen (unpublished results).

Table 5

Nitrate-nitrogen changes in soil as affected by addition of $(NH_4)_2SO_4$ *and crop residues*

Soil treatment	NO_3^- — N, ppm				Increase in NO_3^- — N over soil only, ppm				% apparent nitrification[1]			
	16 °C		27 °C		16° C		27 °C		16 °C		27 °C	
	Weeks		Weeks		Weeks		Weeks		Weeks		Weeks	
	2	6	2	6	2	6	2	6	4	6	4	6
Soil only	25	35	45	57	—	—	—	—	—	—	—	—
Soil + N	38	78	108	130	13	43	53	73*	—	—	—	—
Soil + N + wheat straw	34	118	94	187	9	83	49	130	27	40	36	57
Soil + N + oats	28	119	84	194	3	84	39	137	23	41	43	64
Soil + N + barley	41	130	90	192	16	95	45	135	31	52	31	62
Soil + N + rye	52	135	84	210	27	100	39	153	37	57	36	80
Soil + N + flax	38	137	80	216	13	102	35	159	42	59	58	86
Soil + N + safflower	36	123	73	182	11	88	28	125	19	45	35	52
Soil + N + mustard	37	119	92	217	12	84	47	160	28	41	48	87
Soil + N + rape	49	111	86	209	24	76	41	152	37	33	41	79
Soil + N + peas	35	155	99	229	10	120	54	172	77	77	67	99
Soil + N + alfalfa	27	129	91	200	2	94	46	143	42	61	48	70
Soil + N + grass	38	170	96	211	13	135	51	154	100	92	100	81

* Probably due to denitrification.

[1] No "Apparent Nitrification" took place with crop residues at 2 weeks. Hence data for it is deleted.

Table 6

Nitrate-nitrogen changes in soil as affected by addition of 11—48—0 and crop residues

	NO_3^- — N, ppm				Increase in NO_3^- — N over soil only, ppm				% apparent nitrification[1]			
	16 °C		27 °C		16 °C		27 °C		16 °C		27 °C	
	Weeks		Weeks		Weeks		Weeks		Weeks		Weeks	
	2	6	2	6	2	6	2	6	4	6	4	6
Soil only	25	35	45	57	—	—	—	—	—	—	—	—
Soil + N	43	97	114	135	18	62	69	78*	—	—	—	—
Soil + N + wheat straw	48	148	95	213	23	113	50	156	29	51	48	78
Soil + N + oats	42	144	75	206	17	109	30	149	42	47	43	71
Soil + N + barley	44	152	80	211	19	117	35	154	41	55	42	76
Soil + N + rye	48	151	73	220	23	116	28	163	40	54	42	85
Soil + N + flax	33	162	77	216	8	127	32	159	44	65	55	81
Soil + N + safflower	27	128	68	201	2	93	23	144	14	31	36	66
Soil + N + mustard	47	149	80	222	22	114	35	165	34	52	47	87
Soil + N + rape	54	132	81	219	29	97	36	162	23	35	40	84
Soil + N + peas	48	155	102	235	23	120	67	178	63	58	73	100
Soil + N + alfalfa	47	148	98	203	22	113	63	146	46	51	49	68
Soil + N + grass	43	180	101	223	18	145	66	166	100	83	53	88

* Probably due to denitrification.

[1] No "Apparent Nitrification" took place with crop residues at 2 weeks. Hence data for it are deleted.

It is well agreed upon there is lesser nitrification at below the optimum level of 25—35 °C. Kuo (1955) also observed a similar influence of a temperature of 15 °C on the immobilization of nitrogen. Again, the nature of plant material is obvious in the tables 4, 5 and 6. Russian wild rye grass was nitrified most rapidly but alfalfa did not in spite of the fact that it has almost as much nitrogen as the Russian wild rye grass. This may be due to the presence of some toxic substances in it and this was true with all the 3 nitrogen sources.

Safflower and mustard also showed toxic effects on the nitrification process and seem to delay the mineralization of nitrogen at both the temperatures. This may also be attributed to the nature of the plant materials. Toxic effects of these materials were observed on the CO_2-evolution, as reported by Chandra (1966).

In order to interpret the results in tables 4, 5 and 6 one must notice that the check soil showed a decrease in the "apparent nitrification" which may be most likely due to denitrification starting after a 4-week

Table 7

Statistical analyses for the sources of nitrogen, soil treatments and incubation time for each temperature in Tables 4, 5 and 6*

	16 °C					
Soil treatment	Grass	Peas	Rye	Alfalfa	Flax	Barley
	134	108	99	98	97	93
	Wheat	Oats	Mustard	Rape	Safflower	Soil only
	91	91	90	86	81	68
Sources of N	11—48—0		$(NH_4)_2SO_4$		NH_4OH	
	104		90		90	
Time	6 weeks		4 weeks		2 weeks	
	133		111		40	

	27 °C					
Soil treatment	Grass	Peas	Mustard	Flax	Alfalfa	Rape
	184	182	167	166	162	162
	Rye	Wheat	Oats	Barley	Safflower	Check
	160	160	157	156	148	128
Sources of N	11—48—0		$(NH_4)_2SO_4$		NH_4OH	
	166		159		158	
Time	6 weeks		4 weeks		2 weeks	
	201		193		89	

* Any two means not spanned by the same line are significantly different at the 5% level by Duncan's test.

Any two means not spanned by the line are not significantly different.

period when the nitrification of the added nitrogen was complete. This is particularly important in view of the fact that less nitrogen was mineralized between 4 and 6 weeks in all treatments at 27 °C but there is greater apparent nitrification at 6 weeks. This is so because the data for apparent nitrification are based on the nitrate-nitrogen production in the check soil. There has been more release of nitrogen for mineralization between 2 and 4 weeks in all treatments at 27 °C which indicates a less and less need for the available nitrogen by the heterotrophic microorganisms which are known to prefer ammonia nitrogen and immobilize it in presence of carbon source, Jansson et al. (1955).

Results under laboratory conditions may not reflect upon what actually goes on in a soil under field conditions where the environmental conditions change from day-to-day, but such a study may help to understand the behaviour of a fertilizer under a given set of conditions which may be conducive to know more about the complex nature of nitrogen in soil.

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CHANGES OF THE COMPOSITION OF MICROBIAL COENOSES AND C/N RATIO BY DECOMPOSITION OF PLANT RESIDUES IN THE CHESTNUT SOILS OF THE KULUNDA STEPPE

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As it is generally known, in certain soil types in spite of organic matter which gets into the soil with different carbon and nitrogen contents (litterfall of different plants, straw and other plant residues) a constant quantitative ratio of these elements is to be observed.

This means that the soil is a self-regulating system in which highly precise and effective biological mechanisms, stabilizing the C/N-ratio, function. As it was suggested by Pochon and de Barjac (1960) the action of these mechanisms is related to the losses of carbon as CO_2 during the decomposition of organic matter with high C/N-ratio and nitrogen as NH_3 during the decomposition of organic matter with very low C/N-ratio.

The investigations which were carried out earlier (Klevenskaya and Naplekova 1968) showed that due to the complexity and diversity of organic substrates which are decomposed in the soil, the intensity of microbiological processes and ways leading the C/N-ratio to the definite level were specific for each concrete case.

Our research was aimed at refining our information about the changes in microbe populations and direction of microbiological processes, which by decomposition of different plant residues could get into the chestnut soil of the Kulunda steppe.

The experiments were performed in the percolators mounted according to Macura and Malek's scheme (1958). 250 g of soil and 2 g of dry thoroughly ground plant residues such as wheat straw, young leaves of beet, stalks and leaves of sunflowers, over-ground parts of feather grass, pea, vetch, corn, barley, flax, wormwood were placed into cultivators. The control was the soil, to which plant residues were not added. The incubation of soil and plant residues was carried out under soil moisture of about 20% (that makes up 60% of total moisture), ambient temperature of 20—25° and daily aeration without CO_2.

For 9 months (with periods from 10 to 30 days) the records were kept of ammonificators on meat-and-peptone agar, actinomyces on starch-and-ammonia agar, oligonitrophilic bacteria on Mishustin's medium (1955), denitrificators on fluid Giltay's medium according to Bereosova's modification cellulose-fermenting microorganisms on Hutchinson's medium. Carbon by Knop—Sabanin's method, nitrogen by Modelbauer's method, cellulose by direct method, exchangeable ammonium by Nessler's reagent, nitrates by disulphophenic acid were determined as well. Evolving CO_2 was trapped with 0.1 n $Ba(OH)_2$ followed by titration with 0.1 n oxalic acid. The intensity of biological fixation of nitrogen was determined in special

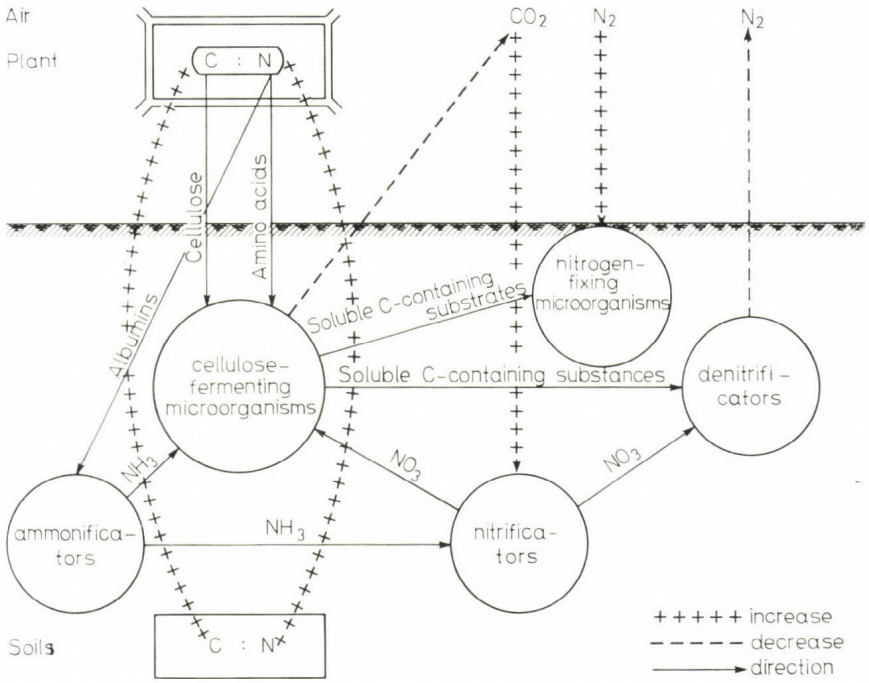

Fig. 1. Principal scheme of microbiological regulation of C/N ratio in chestnut soil

experiments using isotopes. Wide small glasses with 50 g of soil and 0.4 g of plant residues were placed into special desiccators which were filled after pumping with artificial atmosphere of the following composition: nitrogen (enrichment by ^{15}N 93.5 atomic per cent) — 34%, oxygen 21%, CO_2 0.3% and argon 44.7%. The incubation of soil was accomplished as described above. The experiment lasted for 2 weeks. Mass determination of ^{15}N in the soil was carried out by mass-spectrometer MI 1305. When conducting these experiments the number of nitrogen-fixing microorganisms was calculated: azotobacter on Ashby medium, *Clostridium* on Winogradsky's medium and oligonitrophilic microorganisms on Mishustin's medium (1955). Nitrogen-fixing forms were calculated separately among the latters. The determination of nitrogen-fixing ability was done by isotopic and other chemical methods.

The conducted experiments made it possible (following the example of chestnut soil of the Kulunda steppe, to which different organic substrates were applied) to obtain data on the evolution of microbecenoses during the decomposition of plant residues and direction of microbiological processes regulating the C/N-ratio and ascertain some quantitative expressions of the process as well. Principal scheme of microbiological regulation of the C/N-ratio in chestnut soil is shown in Fig. 1. Microbiological equilibrium characteristic of chestnut soil is broken by applying different plant residues to it.

In the early stages of decomposition of plant residues, taxonomic composition of microbe population characteristic of chestnut soil is changed considerably. In microbecenoses the proportion of bacteria especially non-spore-forming ones from *Pseudomonas* increased significantly. The number of actinomyces, especially relative ones decreases, the number of fungi remains almost at the same level increasing considerably only on the very first days of the experiment.

In later stages of decomposition, spore-forming bacteria and *Actinomyces* are developed rapidly. The number of different physiological groups of microorganisms is variable considerably. Interdependent changes in the ratio of the number of microorganisms realizing the metabolism of carbon and nitrogen, become apparent primarily.

The application of plant residues stimulates very much the development of cellulose-fermenting microorganisms which in its turn results in intensive decomposition of cellulose and considerable losses of carbon as CO_2 (Klevenskaya and Naplekova 1968).

The presence of organic substrates containing albumins and amino acids results in an increase in the number of ammonificators and acceleration of the process of ammonification as well. In its turn the former promote the development of the nitrification process which judging from accumulation of nitrates in the soil with applied plant residues proceeds very quickly.

Considerable nitrate storage and also accumulation of soluble carbon-containing substrates (owing to the activity of cellulose-fermenting microorganisms) assure the development of denitrificators which results in losses of nitrogen. To a different extent these losses compensate the activity of nitrogen-fixing microorganisms whose development is caused by the presence of soluble carbon-containing substances as well. The processing of results by statistical analysis showed that there was a direct correlation between the number of nitrogen-fixing microorganisms and intensity of decomposition of cellulose ($r = +0.94 \pm 0.05$). The same correlation was found between the number of nitrogen-fixing bacteria and soil carbon content (for aerobes $r = +0.85 \pm 0.05$, for anaerobes $r = +0.80 \pm 0.11$).

The changes in decomposition of microbecenoses related to the loss of carbon and change of nitrogen content led to the gradual stabilization in the soil of C/N-ratio, disturbed by application of organic residues: the biological equilibrium of the soil is re-established as well.

Evolution of microbe population and rate of re-establishment of microbiological equilibrium and stabilization of C/N-ratio depend on chemical composition of plant residues.

By addition of substrates with high nitrogen storages and low C/N-ratio (for example young leaves of beet, containing 4.1% of nitrogen and C/N-ratio = 7 to chestnut soil cellulose-fermenting microoganisms are developed rapidly which tend to the speedy decomposition of organic matter. The high rate of cellulose decomposition enables intensive development of other processes connected with it, such as nitrification, nitrogen fixation and especially denitrification. The losses of nitrogen by denitrification at early stages of decomposition are not compensated by the activity of nitrogen-fixing organisms notwithstanding that in this case nitrogen fixation proceeds very intensively (Table 1).

Table 1

The change of microbe-cenoses and some chemical indices of chestnut soil on applying plant residues

Indices	Soil without plant residues	Soil + beet leaves			Soil + straw		
		15 days	30 days	60 days	15 days	30 days	60 days
Total number of bacteria, Actinomycetes and fungi per 1 g of soil	1.4	8.0	6.2	22.1	8.0	11.8	10.7
Bacteria, million per 1 g of soil	1.0	7.4	2.5	16.8	7.3	10.1	9.3
Bacteria, %	71.4	92.5	40.3	76.0	91.3	85.3	86.8
Number of Actinomycetes, million per 1 g of soil	0.4	0.6	3.7	5.2	0.7	1.7	1.4
Actinomycetes, %	28.6	7.4	59.2	23.5	8.7	14.4	13.0
Number of fungi, million per 1 g of soil	0.004	0.03	0.03	0.02	0.04	0.04	0.04
Fungi, %	0.3	0.4	0.5	0.09	0.5	0.3	0.4
Cellulose-fermenting microorganisms, million per 1 g of soil	0.07	1.9	0.3	0.5	0.04	0.08	0.02
Oligonitrophilic microorganisms, million per 1 g of soil	2.9	34.4	38.1	25.8	22.3	27.4	16.1
Nitrogen-fixing microorganisms among oligonitrophilic microorganisms, million per 1 g of soil	0.6	3.8	2.6	1.8	1.6	2.2	1.3
Azotobacter, soil crumb percentage covered with bacterial mucus	0	30	not recorded	not recorded	10	not recorded	not recorded
Clostridium in titre	10^{-3}	10^{-5}	not recorded	not recorded	10^{-4}	not recorded	not recorded
Denitrifiers number in titre	10^{-4}	10^{-6}	10^{-6}	10^{-5}	10^{-5}	10^{-5}	10^{-5}

Decomposition degree, % (according to carbon	—	31	34	39	22	34	48
Carbonic losses (as CO_2), mg per 250 g of soil	—	193	210	246	167	271	369
Carbon content, g per 250 g of soil	2.72	3.34	3.10	3.20	3.48	3.20	3.04
Nitrogen content, mg per 250 g of soil	269	344	3.01	324	285	1269	303
NH_4^+, mg per 250 g of soil	3.1	6.9	11.6	5.3	4.7	9.7	4.0
NO_3^-, mg per 250 g of soil	9.2	25.7	64.6	111.0	0	0	40.8
Nitrogen-fixation ^{15}N, atomic per cent		1.040	not recorded	not recorded	0.045	not recorded	not recorded
Nitrogen balance				—19.2			+18.0
C / N ratio	10.4	9.7	10.3	9.9	12.2	11.9	10.0

By adding beet leaves to the soil, a rapid displacement is observed toward the increased relative number of actinomyces (Table 1) which indicates the preliminary stages in the decomposition of organic matter. The high rate of microbiological process assures more rapid stabilization of C/N-ratio.

By addition of chestnut soils of plant residues with less nitrogen content and higher C/N-ratio (for example wheat straw containing 1.3 % of nitrogen and C/N-ratio = 30) the process of decomposition proceeds at first slower than by addition of beet leaves. Then the rate increases and in 30 days the compared versions do not differ in degree of decomposition of applied plant residues. The intensity of denitrification process by addition of straw is not high and apparent losses of nitrogen are not observed. The level of biological fixation of nitrogen is somewhat lower than on addition of beet leaves to the soil.

In the composition of the microbe population the displacement proceeds markedly on the side of increased number of *Actinomyces*.

Due to the slow rate of microbiological process a slower rate of stabilization of C/N-ratio was found as well.

Thus, initial carbon and nitrogen content specific for each species of plants which after dying get into the soil, plays an essential part in the regulation of the rate of stabilization of C/N-ratio in the soil. This regulation is fulfilled by biological mechanisms whose action, in its turn, is caused by requirements of the microorganisms for defined nitrogen and carbon compounds and their actual contents in the medium.

SUMMARY

The soil is a self-regulating system in which highly precise and effective biological mechanisms stabilizing the C/N-ratio function.

Due to the complexity of organic substrates which take place in the decomposition in the soil, the intensity of microbiological processes and ways leading the C/N-ratio to the definite level are specific for each case.

By addition to chestnut soil of the Kulunda steppe, of different plant residues containing different amounts of carbon and nitrogen, microbiological equilibrium characteristic for this soil is disturbed. Interdependent changes in the ratios of microorganism's number indicating the metabolism of carbon and nitrogen become primarily apparent.

In chestnut soil of the Kulunda steppe the principal biological mechanism stabilizing C/N-ratio is decomposition of cellulose by cellulose-fermenting microorganisms. As a result of their activity significant losses of carbon as CO_2 and the process of denitrification occur, the latter gives rise to the losses of reduced nitrogen compounds and partly the process of nitrogen fixation leading to the accumulation of nitrogen.

The initial nitrogen and carbon content in the plants which get into the soil after dying plays an essential part in the regulation of C/N-ratio in the soils and the rate of stabilization. This regulation is performed by biological mechanisms whose action in their turn are induced by requirements of

microorganisms for definite nitrogen and carbon compounds and their actual content in the medium.

The principal scheme of microbiological regulation in the chestnut soil of the Kulunda steppe is given in Fig. 1.

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EFFECT OF DIFFERENT FORMS OF NITROGEN FERTILIZERS ON THE HUMIFICATION OF ^{15}N TAGGED STRAW

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The breakdown of the plant residues introduced into the soil is determined by two opposite processes. One of them is the mineralization process, the other one is the humification which results in the increase of the humic compounds of the soil.

Many experiments have been conducted recently for the study of the humification processes using ^{14}C isotope (Kristanov 1965, Chahal and Wagner 1965, Jansson and Persson 1968, Mutatker and Wagner 1967, Sauerbeck and Führ 1968, Sørensen 1963, Wagner 1968). These experiments demonstrated which part of the plant mass getting into the soil mineralized into $^{14}CO_2$ respectively and how much marked carbon is incorporated into humic materials.

It is well known that besides carbon, nitrogen is the other basic compound of humus. In spite of that there are not many publications dealing with the clarification of the role of nitrogen in humification by means of the isotopic technique (Smirnov 1968, Wójcik-Wojtkowiak 1967, Broadbent 1968, Danneberg et al. 1968, Chu 1966). There has been no publication known to us until now in which the humification process of plant material was studied with ^{15}N isotope.

EXPERIMENTAL PART

To clarify the above listed problems model experiments were conducted in laboratory conditions. Oat straw grond and marked with ^{15}N was introduced into the soil in 5% amount, $NaNO_3$ or $(NH_4)_2CO_3$ were added to the soil in amounts of 1% of straw. Mixtures prepared in this way were incubated for 14, 56 and 112 days in determined temperature and moisture conditions.

The results of the experiments showed (Table 1) that the humification of straw was accompanied by intensive mineralization processes. The added nitrogen made both processes more rapid.

As a result of mineralization the total nitrogen content of the soil and the amount of organic nitrogen decreased and parallel to this the amount of marked mineral nitrogen increased. So, e.g. in the case of treatment 2 containing straw, 93% of the tagged nitrogen remained in the organic bond after 112 days incubation, but in the treatments to which mineral nitrogen war added this amount was only 59—61%. The decrease of the nitrogen in the organic bond was a result partly of the mineralization and partly of the losses of nitrogen occurring during

incubation. In the treatment containing only straw, mineralization amounted to 2.6% and the tagged nitrogen loss was 4% in the treatments containing mineral nitrogen 24—25% from the straw relased and the losses increased to 14—17%.

As the experiments demonstrated the maximal incorporation of the mineral nitrogen compounds introduced into the soil in the course of humification into organic bond took place on the 14th day of the incubation (Fig. 1). It was only after this period that the rate of mineralization surpassed the rate of the incorporation. As a result of this the mineral nitrogen was demonstrable permanently until the end of the experiment. The experimental results which have been published Broadbent and Tyler (1962) as well as Giambiagi and Cerri (1968) are very important. According to their establishments independently of the fact whether nitrogen introduced into the soil was in the form of ammonia or nitrate the majority of nitrogen liberated from the organic material and mineralized secondarily which accumulated into the soil was nitrate nitrogen.

It is evident from the data of Table 1 that the humification of the straw introduced into the soil commenced after mixing. This is proved by the fact that the marked nitrogen can be demonstrated from all the fractions of humic materials after 14 days incubation, though 56—57% of them are incorporated in humic materials insoluble in $Na_4P_2O_7$ and NaOH. With the progress of humification processes the nitrogen amount present in plant materials decreases strongly and parallel with this increases the marked nitrogen occurring in humic and fulvic acid. As regards the nitrogen content of humic and fulvic acid fractions of the treatments containing

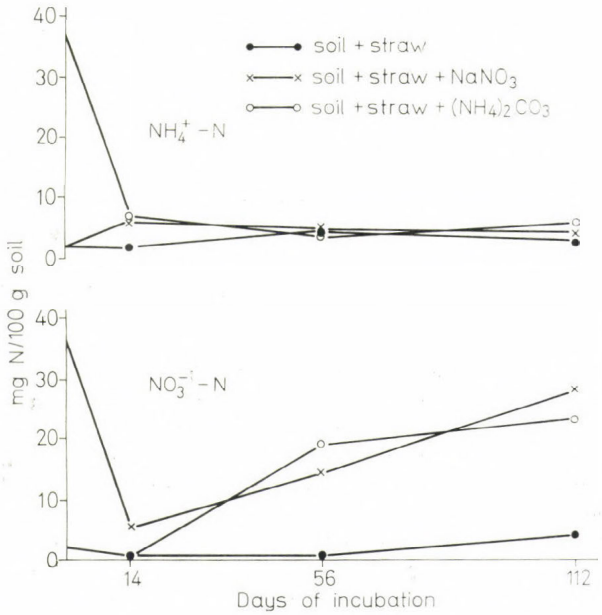


Fig. 1. The change of the quantity of mineral N in the soil during incubation

Table 1

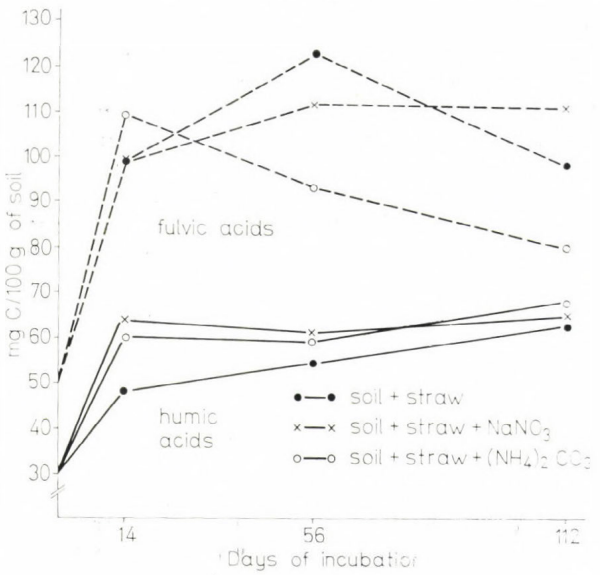
Balance of tagged ^{15}N (in % of straw ^{15}N)

N fraction	Treatment period of incubation, days	Straw + soil								
		without N			+ $NaNO_3$			+ $(NH_4)_2CO_3$		
		14	56	112	14	56	112	14	56	112
$Na_4P_2O_7$ extract	humic acids	4.3	5.1	5.6	5.6	6.6	5.5	6.6	5.5	5.4
	fulvic acids	14.0	18.7	25.0	9.3	16.2	15.2	17.9	13.6	12.2
NaOH extract	humic acids	6.1	6.3	7.0	8.1	8.0	6.9	8.6	8.2	8.3
	fulvic acids	6.1	7.4	6.1	2.4	2.3	3.9	3.1	6.6	4.9
Insouble (humins)		66.8	58.6	49.6	66.5	41.7	27.4	55.8	36.4	29.7
Inorganic		0.4	1.3	2.6	3.5	13.7	23.7	4.5	20.0	25.4
Losses		2.3	2.6	4.1	4.6	11.5	17.4	3.5	9.7	14.1

nitrogen fertilizers, there was no significant difference between the samples taken at the 14th and 114th day of the incubation period.

The constant increase of ^{15}N content of humic and fulvic acids could be observed during the experiment in the treatments having only straw, although the degree of the increase was not high. As an end result there was no essential difference between the humic and fulvic acid content of the different treatments at the end of the 112th day of incubation. These indicate that the humification processes are accompanied by very intensive mineralization. The introduction of nitrogen fertilizers accelerated the humification of straw, but at the same time it stimulated the mineralization of the newly formed humus materials. However, as a matter of fact, the increase of the humic and fulvic acids of the soil was caused by the straw only.

Fig. 2. The change of humic and fulvic acids in $Na_4P_2O_7$ extract



In consequence of straw manuring the total carbon content of

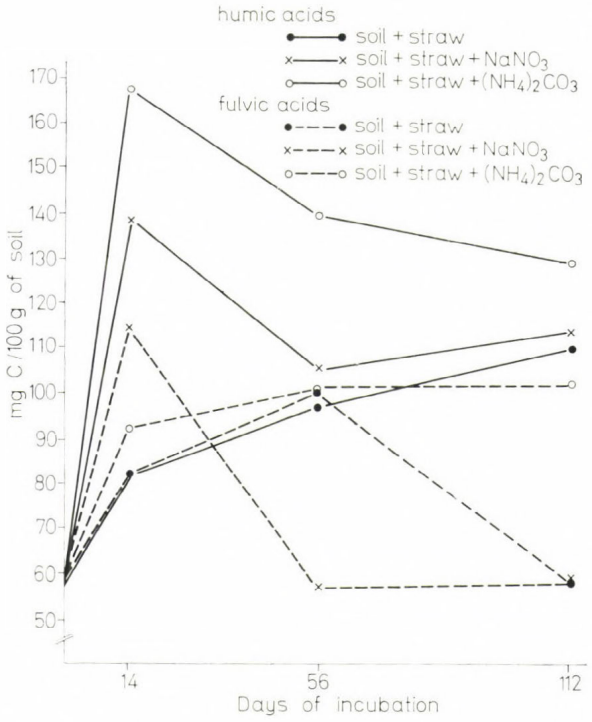


Fig. 3. The change of humic and fulvic acid in NaOH extract

the soil increased. Independent of the big amount of straw added to the soil about 15% of it incorporated into the organic materials of the soil. This fact has been pointed out by many authors too (Chahal and Wagner 1965, Mutatker and Wagner 1967, Wagner 1968). The mixing of the nitrogen fertilizer — primarily the ammonium-nitrogen containing compounds — into the soil though accelerated the mineralization of straw, but after 112 days incubation it does not change the total amount of the humified carbon content of the soil.

The amount of the humic and fulvic acids can be extracted with $Na_4P_2O_7$ as well as NaOH changed in the investigated soils in the course of incubation. Both humus fractions increased by double compared with organic matter content of the original soil in the first 14 days of incubation. In the $Na_4P_2O_7$ solution the fulvic acids were dominant, in the NaOH extract the humic acids with the time of incubation. The amount of both humus fractions decreased intensively in all variants of the experiments (Figs 2, 3). It can be concluded from this that the humic compounds formed newly are not lasting and they are easily mineralized. It concerns firstly the humic compounds which formed after addition of nitrogen. Between the humic fractions received with the two extractive methods especially the fraction soluble in NaOH mineralized strongly, while the humic acids obtained

Table 2

C/N ratio in the humic and fulvic acids

Fraction	Control soil	after 1,12 days incubation		
		soil + strow		
		without N	+ $NaNO_3$	+ $(NH_4)_2CO_2$
Humic acids	19.9	12.6	11.3	10.4
Fulvic acids	9.5	8.6	8.1	9.5

with $Na_4P_2O_7$ are more resistant. An essential change was noticed during the examination as regards the C/N ratios (Table 2). In the case of the control soil the fulvic acids contained much nitrogen and their C/N ratio did not change on the effect of incubation or different treatments. In spite of that during incubation the nitrogen content of humic acids increased significantly independently of the fact whether it was treated with nitrogen or only straw was introduced into the soil.

SUMMARY

The humification of straw introduced into the soil started with intensive mineralization process just after it was mixed into the soil.

The nitrogen fertilizers increased at the same time as the humification and mineralization process. There is no essential deviation concerning the effect of ammonia as well as nitrate nitrogen in the two processes.

The nitrogen fertilizers combined into organic bond especially during the first 14 days of the incubation. After this the mineral nitrogen could be detected in the soil, firstly as NO_3^-. The nitrogen in the straw has a direct role in the synthesis of the different fractions of humic materials. The humic materials formed newly, mineralized intensively especially in the treatments with mineral nitrogen. Moreover the incorporation of nitrogen fertilizers did not increase the humic content of soils.

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MICROFUNGI ISOLATED FROM *PINUS RADIATA* LEAF-LITTER

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Different studies have already been conducted on the fungal flora accompanying the decay of conifer leaf-litter, particularly of some pine species.

Because of interest in *Pinus radiata* fungal environment, we have carried out — as an extension of previous researches — some investigations on microfungi inhabiting the upper (horizon A_0) and lower (horizon F) litter of this conifer. As it is known, radiata pine is a 'fast growing' species, which is of particular interest to the cellulose industry.

MATERIALS AND METHODS

In spring 1959 samples were removed from three different sites, i.e.:

Station №. 1 — Locality: Passo dello Scopetone (Arezzo, Tuscany) at some 500 m a. s. l. A not too large area covered with trees since about 37 years. Litter thick up to 11 cm on loam texture; rocky layer occasionally emerging; no undergrowth.

Station №. 2 — Locality: Casalotti (Rome). Experimental plot, at sea level, constituted by some 1 000 trees 7 years old. Loam texture, rather clay like; 6—7 cm thick leaf-litter; no undergrowth.

Station №. 3 — Locality: Oristano (Cagliari, Sardinia). Experimental plot at sea level constituted by some 2 000 trees 6 years old. Loam texture with a fair content of sand; 4—5 cm thick litter; no undergrowth.

The various samples, removed from different spots of each station, were finely ground until a homogeneous powder was obtained. The samples from each site were then carefully mixed together, in order to have a sample as representative as possible of each station.

Later on, 1 g of each sample was introduced in 1 000 ml of sterile water; then, 10 Petri dishes were inoculated with 0.1 ml of suspension and incubated for 6—12 days at 24 °C with Difco mycological-agar. After 6 and 12 days, isolations were performed of all colonies present in the 10 dishes. The resulting number can so be regarded as the amount of fungi existing in 1 ml of suspension of 1 g of litter in 1 000 ml of water, i.e. 1 mg of litter.

As concerns isolations, Czapek-agar was used for *Penicillium* and *Aspergillus*, and mycological-agar for the other species.

RESULTS AND DISCUSSION

1. Isolations conducted in the station №. 1 (Passo dello Scopetone):
   Ao (upper litter); 11 strains.

Alternaria sp. 1; *Alternaria* sp. 2; *Rhinocladiella* sp.; *Fusarium* sp. 1; *Geotrichum candidum* Link.; *Cladosporium herbarum* (Pers.) Link.; *Cladosporium* sp. ; *Aspergillus versicolor* (Vuill.) Tiraboschi; *Penicillium janthinellum* Biourge; sterile mycelium №. 1; sterile mycelium №. 2.

F (lower litter); 51 strains.
Mucor sp.; *Trichoderma lignorum* (Tode) Harz. (5 strains); *Cheiromyces stellatum; Rhizopus* sp. (6 strains); *Cephalosporium acremonium* Cda (3 strains); *Cladosporium* sp.; *Epicoccum nigrum* Link (2 strains); *Penicillium chrysogenum* Thom; *Pen. janthinellum* Biourge (9 strains); *Pen. sclerotiorum* van Beyma; *Pen. corylophilum* Dierckx (3 strains); *Pen. cyclopium* Westling (3 strains); *Pen. lilacinum* Thom; *Pen. restrictum* Gilman et Abbott; *Pen. frequentans* Westling; *Pen. roseum-purpureum* Dierckx (2 strains); not-fructifying mycelium (10 strains).

At Passo dello Scopetone the fungal flora appears, therefore, very differently distributed between the upper and lower layer of the leaf-litter. Common to the two layers were only some species of *Cladosporium*, *Pen. janthinellum* and not fructifying mycelia, the latter being particularly abundant in F. This probably proves the extremely different conditions of moisture, temperature and light between the two layers.

2. Isolations conducted in the station №. 2 (Casalotti)
A_0 (upper litter); 66 strains.
Cladosporium sp. №. 1; *Cladosporium* sp. №. 2; *Cladosporium* sp. №. 3 (2 strains); *Stemphylium* sp. (5 strains); *Pyrenochaeta decipiens* March. (23 strains); *Penicillium* sp; yeast №. 1; yeast №. 2; not fructifying mycelium №. 1, №. 2, №. 3, №. 4 (3 strains).

F (lower litter); 261 strains.
Cladosporium sp. №. 1 (19 strains); *Cladosporium* sp. №. 2 (2 strains); *Pestalotia macrotricha* (3 strains); *Alternaria* sp. (2 strains); *Pyrenochaeta decipiens* March. (57 strains); *Fusarium* sp. №. 1 (3 strains); *Fusarium* sp. №. 2 (3 strains); *Rhizopus* sp. (2 strains); *Pen. frequentans* Westling (4 trains); *Pen. chrysogenum* Thom (4 strains); *Pen. citricum* Thom; *Pen. lilacinum* Thom; not fructifying mycelium №. 1, №. 2, №. 3 (55 strains); №. 4 (10 strains); №. 5 (14 strains); №. 6 (7 strains); № 7 (4 strains); №. 8 (68 strains).

The Casalotti station results proved to be considerably richer, both quantitatively and qualitatively, than the preceding one. However, there is always the same great numerical difference between the layer A_0 and F.

From the point of view of the species, there exists conversely a certain similarity between A_0 and F, as it is demonstrated by the larger presence in the two horizons of *Pyrenochaeta decipiens*, different *Cladosporium* and not fructifying mycelia, these being in greater amount in F than in A_0.

3. Isolations conducted in the station №. 3 (Oristano)
A_0 (upper litter); 13 strains.
Cephalosporium sp.; *Pen. cyclopium* Westling; not fructifying mycelium no. 1; not fructifying mycelium №. 2 (10 strains).

F (lower litter); 278 strains.
Cephalosporium sp. №. 1 (8 strains); *Cladosporium* sp. (31 strains);

Stachybotrys atra Cda; *Rhizopus* sp. (2 strains); *Stemphylium* sp. (13 strains); *Trichoderma lignorum* (Tode) Harz.; *Hormodendron* sp. (3 strains); *Alternaria* sp.; *Pen. cyclopium* Westling (15 strains); *Pen. frequentans* Westling (10 strains); *Pen. janthinellum* Biourge (2 strains); *Pen. decumbens* Thom (6 strains); *Pen. fellutanum* Biourge (2 strains); *Pen. waksmannii* Zaleski; *Pen. corylophilum* Dierckx (2 strains); *Pen. chrysogenum* Thom (2 strains); *Pen. lilacinum* Thom (2 strains); not fructifying mycelium №. 1 (41 strains); №. 2 (4 strains); №. 3 (2 strains); №. 4 (70 strains); №. 5 (17 strains); №. 6 (16 strains); №. 7 (8 strains); №. 8 (20 strains).

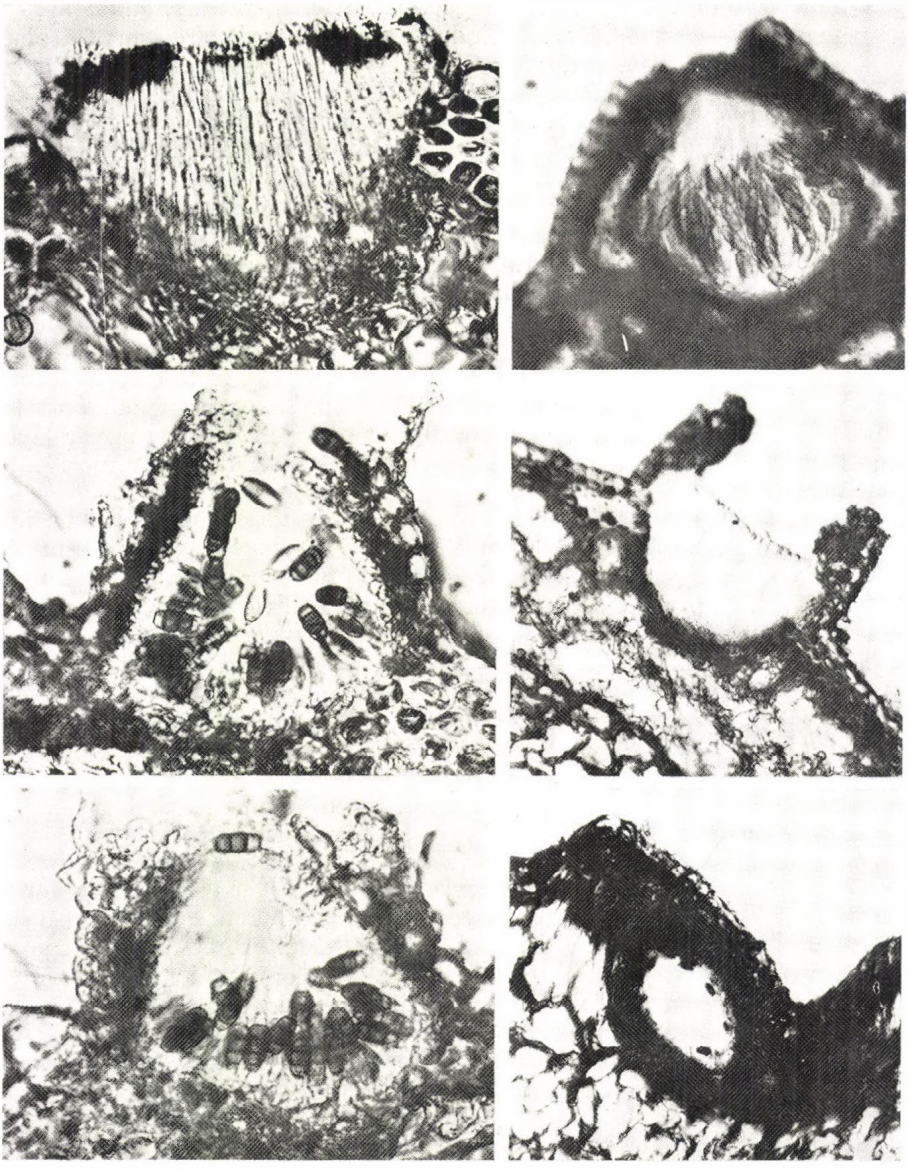
From the point of view of the quantitative relations, this station does not differ from station №. 2, that of Casalotti. On the contrary, a great difference exists between the two stations as concerns the ratio A_0/F, the upper layer at Oristano proving that there were hardly any species. There is not doubt, this is due to the peculiar characteristics of the environment, namely the high temperatures which are sometimes attained, and the almost constant winds, implying conditions of aridity in the upper layer. This fact is also proved by the lower horizont with its great number of Penicillia.

Finally, as to the species distribution, the results cannot be taken into consideration, because of the small number of species observed in A_0.

As far as we know, no research has been so far conducted on microfungi taking part in *P. radiata* leaf-litter decay. The investigations concern in fact the litter of *P. sylvestris* L. and *P. nigra* spp. *laricio* (Poir.) P. Corte in England (Ward, 1952); of *P. sylvestris* in Holland (Gremmen 1957); of *P. sylvestris* in England again (Kendrick & Burges 1962, Kendrick 1963, Hayes 1965); and finally of *P. monticola* Dougl. and *P. ponderosa* Dougl. in Kansas (Brandsberg 1969).

In spite of the extremely different results obtained, as to fungal flora, all the authors seem to agree upon the differences existing between the upper and the lower layer of the coniferous leaf-litters investigated; fungal similar differences seem also to exist between microfungi of first colonization and those developing later; discrepancies can be finally found in relation to the environment. No considerable differences are conversely observed between the fungal flora of conifer litters, as also between conifer litters and those from other plants. The results obtained mostly confirm such conclusions. In fact, also from our investigations it appears that the horizon A_0 considerably differs (mainly quantitatively) from the layers below; differences may also be found between microfungi of first colonization and those developing later, as also discrepancies occur in relation to the environment. In this respect, our data prove significant enough; the leaf-litter collected in the first station (Scopetone), located on a hill at 500 m a. s. l., shows in fact a number and a variety of species lower than in the leaf-litters of the stations situated at sea level. As concerns the horizon A_0, discrepancies are also observed between the two stations of Oristano and Casalotti, this layer being less provided with species in the former than in the latter plot, owing to the presence of a drier environment for both lower precipitations and more constant winds.

Through the few data collected it is certainly not possible to establish whether the fungal flora of radiata pine leaf-litter differs or not from that of other pine species. As far as we can deduce from our studies and as far

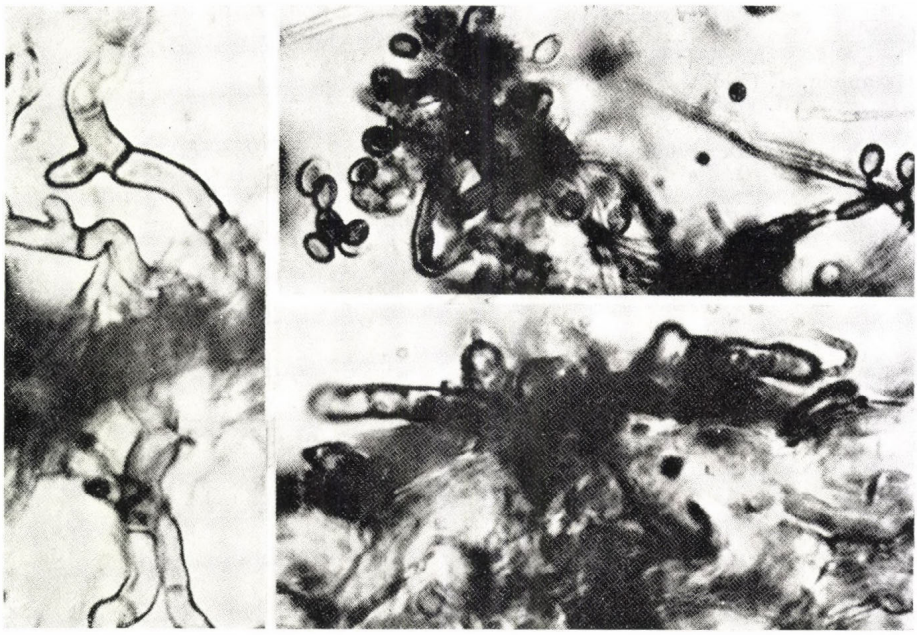


a

Fig. 1/*a* and *b*. Microscopical sections of *Pinus* needles

as the above-mentioned authors have proved for other pine species, we are inclined to affirm that probably no significant differences will be observed.

We think the coniferous leaf-litter, and particularly that of pine, is sufficiently featured by microfungi (including also Spheriaceae and Discomycetes) which quite often colonize the needles to some extent when the latter are going to fall and, to a considerable extent, soon after they fall



b

to the soil. This is proved on examining the needles with the naked eye or, still better, by means of microscopical sections (see Fig. 1). Perhaps this aspect of the first colonization of the needle-litter, mainly by soil not-inhabiting species, has so far not been sufficiently emphasized.

It is needless to point out the active role played in litter decomposition by Basidiomycetes, mostly represented in culture by strains indicated as sterile or not fructifying mycelia. Among these higher fungi we have frequently observed *Clitocybe olearia*, *Boletus granulatus*, *Boletus queleti*, *Hypholoma fasciculare*, *Mycena galopoda*, *Mucidula mucida*, etc.

SUMMARY

Investigations were carried out on the fungal flora of *Pinus radiata* leaf-litter in three ecologically different stations of central Italy and Sardinia.

Isolations were made from both upper and lower layers. Few species, varying from station to station, were observed in the upper layer, while numerous species were found in the lower horizon. In the latter, different species proved to be present in all three stations, thus showing that they were not affected by environmental factors. These, on the contrary, seem to exert their action on the quantitative aspect only.

When considering the *isolated species* as taking part in litter decay, it is necessary not to neglect the destructive role often played by the micro-fungi *already inhabiting the needles*, and which — as the present work points out — have already colonized and in many cases fructified both outside and inside the tissues.

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MICROBIAL DECOMPOSITION OF ORGANIC MATTER UNDER NATURAL CONDITIONS

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The PT section of the International Biological Program has very important tasks in the primary production of higher plants under natural conditions. However, in this report we will be concerned with the decomposition and the metabolism of organic matter which is known as the secondary production.

How is the organic matter distributed under forest soils supplying the nutritional substances for microbial growth? The decomposition of organic matter has been studied both quantitatively and qualitatively in detail. It is well known that the organic matter in soils is subjected to decomposition by microorganisms. How are they produced and how do they evolve carbon dioxide as the final products?

The purpose of this report is to give an account of the secondary production and the decomposition processes related to soil microorganisms under the sub-alpine type pine forest of *Tsuga-Abies* forest in Japan.

The following points have been examined: I. The properties and the metabolism of organic matter in these forest soils. 2. The behaviour and activity of litter decomposing microorganisms. 3. The microbial ecology of these forest soils and the microbial decomposition processes in these experiments.

MATERIAL AND METHODS

Soils and litter samples ware taken from each point identified as wet podzolic soil, Pw, dry podzolic soil, Pd; and humid brown soil, Bd. These samples were collected in June and October in 1968 respectively.

The JIBP (International Biological Program for Japan) area investigated was classified as wet podzolic soils mostly covered with *Tsuga-Abies*. The heterogeneity of this area was clearly recognized as regards vegetation and soils.

The soil pH and moisture content were measured with Beckman pH meter Model GS and the ignition method. For total carbon and total nitrogen analysis the sulphuric acid digestion method and the micro-Kjeldahl method were used. The organic matter was estimated with the proximate method modified by Waksman's.

The carbon dioxide was measured with the trapping bottle method. The microbial counting and tests were carried out on bacteria and moulds with generally used techniques (Table 1).

RESULTS AND DISCUSSIONS

The JIBP-area is situated at 1,760 m high (above sea level) in central Japan. The soils here originate from andesites. The vegetation is covered with *Tsuga-Abies* forest and partially *Betula*. Podzolization proceeded in this area under such conditions. Soil surveys were carried out according to three points in the following markings, Pw, Pd and Bd (Table 2).

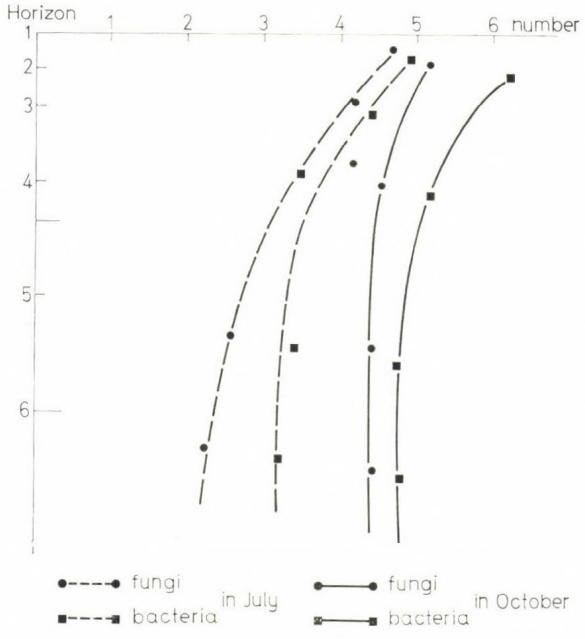
Pw: wet podzolic soil

The A_1 layer is not clear, B_1 layer (below) consisted of tuff, and B layer (above) was covered with volcanic ashes. Each of F, FH and H layers over A_0 layer was thick, especially H layer had 10 cm thickness. This is identical with humid soil character. The A_2 layer revealed the leached zone. It was of a greyish colour, and had many cracks. Depending on the high content of humus, the organic matters become leached, then the Bh layer humified. The B_1 layer had a compact pan structure. This structure demonstrated that the wall structure depended on the high moisture content (Fig. 1).

Pd: dry podzolic soil

These types of soils are found on dried and well drained points. There are many gravels mixed up with plant roots. It is very difficult to recognize the individual horizons owing to their less sharp boundaries. It is characterized in the H layer. This soil has the crumb structure according to the well drained state (Fig. 2).

Fig. 1. Distribution pattern of microbes at the point of D_5 profile (Pw)



Bd: humid brown forest soil

This type of soil is different from the above mentioned soils, Pw and Pd. This was classified as the brown forest soil, but it has a clear iron leached zone in the B layer. Therefore it is considered that some degree of podzolization had occurred. Compared with both Pw and Pd soils, the A layer of Bd soil was clearly recognized, but the thick humus accumulated, and had a high moisture content (Fig. 3).

Fig. 2. Distribution pattern of microbes at the point of F_4 profile (Pd)

pH and moisture content

It is considered that the low pH indicated that these soils have podsolized. The pH of Bd was the same as on Pw and Pd soils. Moreover this Bd soil had been affected by podzolization in the past.

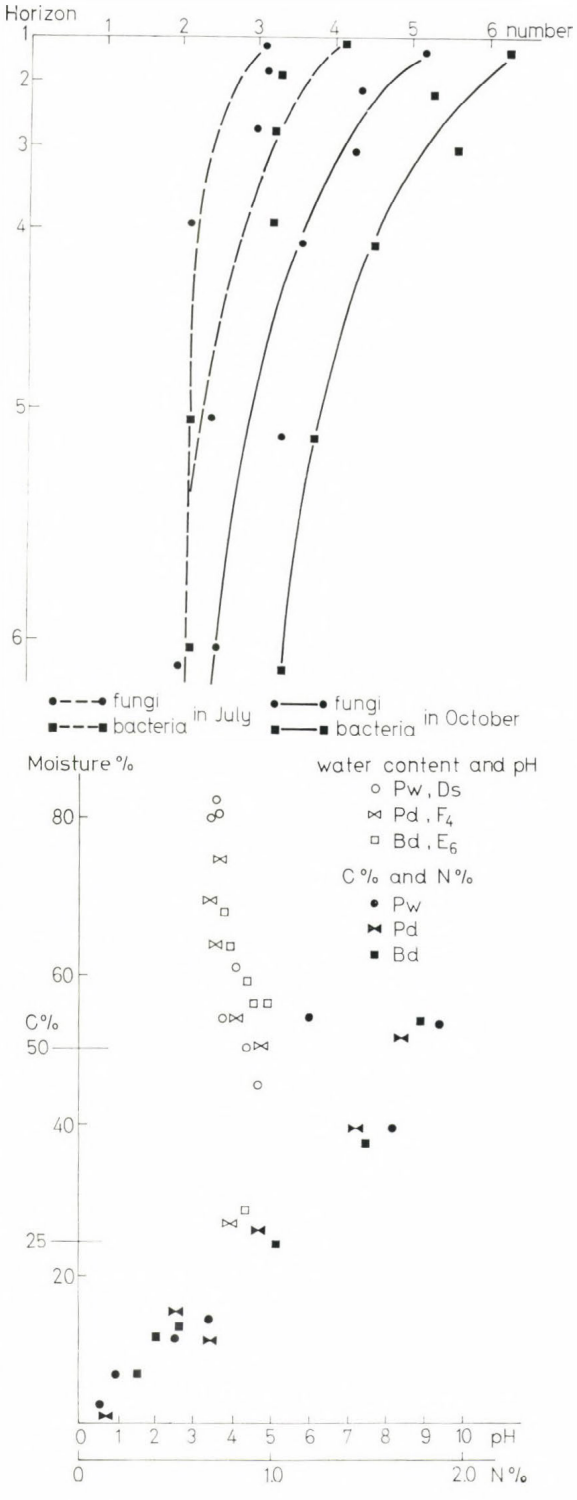
From these observations one can conclude that the leaching of cations and the formation of humus have taken place. Comparing each layer, the pH values indicated a minimum at H and A layers respectively and then increase their values a little. It could be said that in these layers, the degradation has proceeded more rapidly and afterwards the acid humus formed on these layers.

The moisture content in Pw fresh soils, especially at A_0 layer showed over 80%, in Pd rather higher content. However, both Pw and Pd have been characteristically recognized by means of moisture contents.

Total carbon and nitrogen and C/N ratio (Fig. 4)

The C/N ratio showed the maximum at A_0 layer. It is deduced to the volume of organic matter

Fig. 3. Distribution pattern of microbes at the point of E_5 profile (Bd)



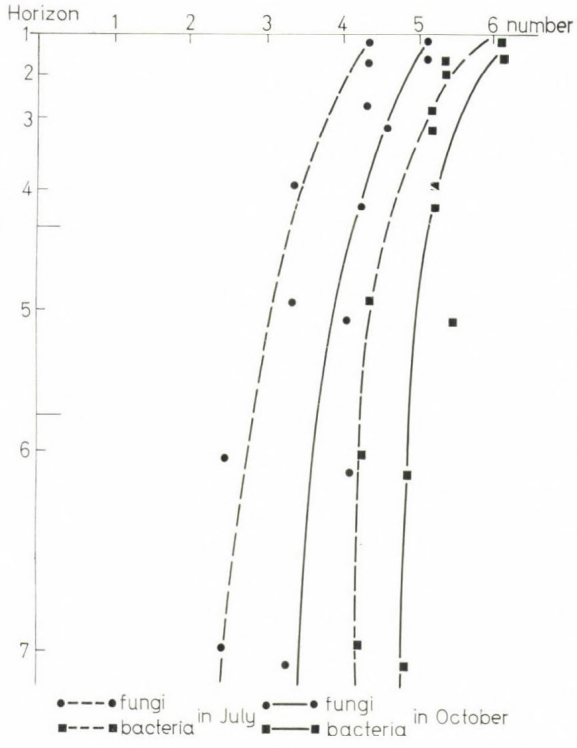


Fig. 4. Relationship between water content, total N and C

supplied by litters. The pine litter itself is difficult to decompose or resistant to decomposition under these conditions. The C/N ratio has a rather high value at A and B layers generally. This trend is a common characteristic under the forest zones, and some parts depend upon the leaching or the decomposition of organic matter and humus.

Comparing Pw and Pd on the total carbon and total nitrogen, Pd soils have shown a great decomposition rate. This trend was also certified by the carbon dioxide evolution (Table 4).

Microbial decomposition of organic matter

The H_2SO_4-insoluble fraction remained as the most resistant part followed by the HCl fractions. The H_2SO_4-soluble, hot-water-soluble and alcohol-benzene-soluble fractions had the same values (Table 3).

It could be said that the H_2SO_4-insoluble fraction was the most stable or resistant to microbial decomposition. It is recognized that in each layer some unequal decomposition has occurred, and the upper layers are affected by microbial activities mostly by *Penicillium* and *Bacillus* during the spring-summer-fall seasons.

It can be concluded that the decomposition processes at the A_0 layer showed the different distribution of organic matters for each sample. Not enough experiments have been done on the decomposition and distribution under natural conditions, because this area has such heterogeneous characteristics in organic matter and the microbial activities have been limited by environmental factors.

SUMMARY

This report deals with the following points: 1. The nature of soil organic matter and its metabolism. 2. Behaviour and activity of microorganisms under the subalpine type pine forest. 3. The decomposition processes in forest soil.

Table 1
Media used for the experiment

Meat Peptone Agar	
Meat Extract	10 g
Peptone	10 g
Glucose	5 g
Agar	20 g
pH = 6.8	
Malt Glucose Yeast Agar	
Glucose	10 g
Yeast Extract	10 g
Malt Extract	10 g
Agar	20 g
pH = 6.8	
Rose Bengal Agar	
Glucose	10 g
$NaNO_3$	1 g
K_2HPO_4	1 g
Agar	15 g
Rose Bengal	1/15000
Potato Extract	1000 ml
pH = 5.0	
Albumin Agar	
Glucose	1 g
K_2HPO_4	0.5 g
$MgSO_4 \cdot 7\ H_2O$	0.2 g
$FeSO_4 \cdot 7\ H_2O$	0.01 g
Egg Albumin	0.25 g
Agar	15 g
pH = 6.8	
Thioglycollate Agar	
Meat Extract	10 g
Peptone	10 g
Na-thioglycollate	2.5 g
Agar	15 g
pH = 6.8	
Eggins-Pugh Agar	
$(NH_4)_2SO_4$	0.5 g
L-Asparagine	0.5 g
KH_2PO_4	1.0 g
KCl	0.5 g
$MgSO_4 \cdot 7\ H_2O$	0.2 g
$CaCl_2$	0.1 g
Yeast Extract	0.5 g
Cellulose	10 g
pH = 6.2	

Sampling was done in the JIBP-area at Shiga-yama Heights. Samples were collected from three types of soils which were identified as 1) wet podzolic soil, Pw; 2) dry podzolic soil, Pd; and 3) humid brown forest soil, Bd respectivelly.

These samples were taken from different layers in June and October 1968. Strong acidity was predominant in all samples at about 4.0 pH. It is recognized that the formation of humus and the leaching of cations proceed in the upper layers of the soil. A lot of heterogeneous characteristics in mountainous forest area as regards geography, soils and vegetation have been found.

The carbon-nitrogen ratio indicated about 30 in all samples except wet podzolic soil which showed over 40. It is suggested that the accumulation processes proceeded rapidly compared with the decomposition processes under *Tsuga-Abies* forest. This points to the influence of environmental heterogeneity on the distribution of microorganisms in this area.

The litter decomposing microorganisms were predominant in the fall season in September. However, the carbon dioxide evolved remarkably after snow thawing in May. It has been said that the distribution of microorganisms in each layer was quite constant in each season under these conditions. This phenomenon demonstrates the tight relationship between

Table 2

General characteristics of soil samples

Soil type	Horizon	Depth, cm	Texture	Moisture, %	pH(H_2O)	C%	N%	C/N
Pw	F	0— 5		80.6	3.7	34.3	1.40	38.8
	FH	5—11		81.7	3.7	53.3	1.90	28.8
	H	11—22	LC	80.4	3.5	39.4	1.65	23.9
	A_2	22—29	LC	54.2	3.9	14.1	0.67	21.0
	Bh	29—33	LC	60.6	4.1	12.5	0.49	25.6
	Bi	33—36	LC	50.0	4.5	5.2	0.19	27.1
	C	36—52	SC	45.3	4.6	2.6	0.13	20.1
Pd	F	0— 3		74.8	3.8	51.1	1.71	29.9
	MFH	3— 8		69.9	3.5	39.7	1.48	26.9
	A	8—15	CL	54.2	4.1	15.2	0.56	27.1
	Bi	15—23	CL	49.9	4.6	12.8	0.71	18.0
	B	23—38	SL	26.2	4.8	3.3	0.14	23.6
Bd	L			29.2	4.3	53.1	1.82	29.2
	F	0— 3		68.2	3.9	38.4	1.53	25.2
	H	3— 9	CL	63.1	3.8	25.7	1.05	24.4
	A	9—15	CL	59.2	4.2	13.7	0.53	25.8
	B_1	15—23	CL	56.5	4.5	12.0	0.40	30.0
	B_2	23—38	CL	56.9	4.8	8.5	0.32	26.6

Table 3

Proximate analysis of organic matter in A_0 and A_1 layers

Soil type	Horizon	Alcohol-benzene extract Lipid, Resin	Hot water extract organic matter, Sugars	2% HCl extract, Hemicellulose	72% H_2SO_4 extract, Cellulose	Residue, Lignin
Pw	F	5.1	5.4	8.3	4.8	45.3
	FH	5.2	5.5	8.4	7.0	45.4
	H	1.9	3.9	4.8	1.2	22.3
	A_2	1.3	1.6	1.1	0.6	6.9
Pd	F	6.7	6.0	11.0	5.9	43.3
	MFH	5.0	4.7	8.8	4.6	40.4
	A	4.1	2.1	2.2	1.0	17.8
Bd	L	5.7	7.3	13.6	13.3	46.2
	F	3.8	4.6	10.0	4.3	37.6
	H	2.0	2.4	4.7	1.3	12.2
	A	0.6	0.8	2.1	0.2	7.2

+ % dried soil

space and time to microbial distribution. The presence of stable distribution patterns of microorganisms through each season in the *Tsuga-Abies* forest soils could thus be concluded.

Table 4

Evolution of CO_2—C mg from various soils in JIBP-area

Soil type	Horizon	Moisture, %	Carbon, %	mg/dry soil	C% wet soil	C % dry soil	C %/month
Pw	F	75.7	51.7	9.0	37.2	72.0	2.2
	FH	79.6	51.0	3.3	16.3	32.0	1.0
	H	74.1	32.5	0.8	3.0	9.2	0.3
	A	61.0	15.7	0.2	0.2	3.9	0.1
Pd	F	33.4	52.7	8.7	13.0	24.7	0.7
	MFH	71.5	47.4	4.3	15.1	31.9	1.0
	A	65.9	20.9	2.1	6.0	28.9	0.9
Bd	F	76.5	44.2	4.4	18.8	42.5	1.3
	H	68.6	27.8	1.3	4.1	14.6	0.4
	A	54.0	12.3	0.3	0.7	5.9	0.2

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HUMIFICATION AND MINERALIZATION OF PLANT REMAINS AS STEPS OF ECOSYSTEM REGULATION IN ÑADI SOILS OF SOUTHERN CHILE

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In Chile, the oceanic climate of the southern central zone allows the biological activity to extend over almost the whole of the year. The dominant elements of the climatic temperate rainforest biocenosis are of a hygrophytic and mesothermic type (di Castri 1964).

In fact, the forests on hydromorphic soils derived from volcanic ash (ñadis) are part of a subactual ecosystem with exceptionally efficient regulation. They belong to a *Nothofago-Eucryphion* with endemic subtropical components, in addition to antarctic species which are relicts from the last glaciation.

The survival of this laurel-leaved evergreen "Valdivian forest" is not only due to high relative humidity and absence of frost, but also to its tolerance to occasional summer drought and to the perpetuation of a particular microclimate which maintains this formation in an environment having undergone considerable change.

The regional climate is characterized by a mean temperature of 10°C (6.5°—14.8°C), a relative humidity of 82% (76—87), a rainfall around 1700 mm. distributed as follows: 30% in autumn, 35% in winter, 20% in spring, 15% in summer.

The presence of allophane, amorphous clay derived from volcanic glass, commands the metabolic activity of ñadi soils. The positive charges of this clay are responsible for the very active adsorption of organic anions, as fulvic acids. Humification is proceeding normally but mineralization is thus slowed down; considerable accumulation of organic matter takes place (4). Fulvic acids are involved in a feed-back regulation mechanism of the ñadi ecosystem (2); they are key metabolites both as concerning energy-flow and complexing of high amounts of aluminium (3).

It was essential to explore the patterns of humification, as proceeding under controlled conditions of incubation, of dead leaves collected from the most representative species of the climatic woodland of ñadis.

MATERIAL AND METHODS

We present in this paper part of the results concerning the three evergreen species which are dominant in the biomass of the Valdivian temperate rain forest:

"Coigue": *Nothofagus dombeyi* (Mirb.) Bl.: Fagaceae
"Radal": *Lomatia hirsuta* (Lam.) Diels: Proteaceae

"Canelo": *Drymis winteri* Forest. var. *chilensis* (D.C.) A. Gray: Winteraceae (Magnoliales).

Fallen leaves were collected on nylon nets monthly over a yearly cycle, dried, pooled and milled to 500 μ. 5 g were incubated 50 days at 28°C in 500 ml Erlenmeyer flasks at field capacity (20 ml), plugged with cotton, in order to provide optimal aerobic conditions. Anaerobiosis was achieved at saturation (30 ml) under N_2-atmosphere. Every 10 days one flask was analyzed as follows:

a) aqueous extract (1): repeated extraction with portions of a total of 50 ml of water at 20 °C.
b) Soxhlet extract with ether.
c) Aqueous extract (2): as under (a).
d) Lignocellulose: residue treated with H_2SO_4 5% for 3 h at 100°, centrifuged, washed.
e) Hemicellulose: reducing power of solution from (d).
f) Lignin: residue treated with H_2SO_4 72% for 4 h at 0°C, then diluted to 23 vol. with water and heated 3 h at 100°C. Centrifuged, washed.
g) Cellulose: reducing power of solution from (f).
h) Ash: lignocellulose and cellulose.

In a second series we extracted every 10 days, obtaining an aqueous extract 1), as in the first series. The material was then reincubated, reinoculated with 1 ml of soil suspension 10^{-3}.

In both series the CO_2-evolution was measured on the second day, then every 10 days, extracting the CO_2 under depression and receiving $Ba(OH)_2$.

Reducing power was measured according to Hagedorn—Jensen: reduction of ferricyanide, titration with 0.02 N thiosulphate.

RESULTS

It may be seen that the three species differ largely as regards *composition* (Tables 1—2): *Nothofagus* is very high in soluble matter, with high reducing power, high fulvic acid content and very low pH of the extract, low in lignin. *Lomatia*, on the contrary, is very high in lignin and lower in all other fractions. *Drymis* is low in soluble matter and reducing power.

Table 1
Initial material (100 g): distribution of fractions

Species	H_2O extract 1	Ether extract	H_2O extract 2	Hemi-cellulose	Cellulose	Ash cellulose	Lignin	Ash Lignin	Total ash
Nothofagus	16.55	6.01	6.09	35.47	14.81	0.07	20.81	0.19	4.33
Lomatia	13.46	5.32	2.49	23.51	11.15	0.04	43.81	0.22	3.74
Drymis	7.48	5.06	2.41	33.66	13.96	0.06	37.07	0.30	4.23

From the *reincubation* experiment, which indicates the potential release of soluble matter, reducing power, fulvic and humic acid fraction as controlled by aerobic or anaerobic conditions during 60 days of incubation, it may be concluded (Table 3) that *Nothofagus* again stands far above

Table 2

Initial material (100 g): reducing power of water extracts and of hemicellulose fraction, and pH of water extract

Species	Reducing power					pH	
	Aqueous extract		Fulvic acid	Humic acid	Hemicellulose	Aqueous extract	
	1	2	1	1		1	2
Nothofagus	4347	1884	3501	523	8918	4.1	4.6
Lomatia	2369	596	2208	120	5343	5.4	5.1
Drymis	1520	554	1400	80	9144	5.4	5.4

Table 3

Cumulative loss of soluble matter and reducing power, per 100 g of dry material incubated (28°C) and extracted every 10 days with water

Species	Days of incubation	Under aerobiosis					Under anaerobiosis				
		H_2O extract	pH	Reducing power			H_2O extract	pH	Reducing power		
				Total	Fulvic acid	Humic acid			Total	Fulvic acid	Humic acid
Nothofagus	10	16.6	4.0	4370	3812	493	17.4	4.1	4832	4077	566
	60	26.4	4.7	6227	5283	827	26.1	4.3	6581	5567	735
Lomatia	10	9.0	4.7	723	603	90	10.5	4.4	773	667	70
	60	12.8	5.8	1482	1135	231	16.5	4.8	1643	1264	275
Drymis	10	8.4	6.5	845	650	97	9.2	5.5	1170	930	120
	60	13.5	6.1	1723	1281	312	14.8	5.7	2054	1591	284

the other two species. It is interesting to note a temporary low, after 10 days in the fulvic acid fraction from *Lomatia* and *Drymis*. Anaerobic conditions lead, in general, to increased values; organic acids, among other metabolites, accumulate and maintain a low pH.

Soluble matter of *Nothofagus* is abundant and corresponds to a high value of reducing power which moreover increased constantly. The results concerning the two other species are much lower and comparable, with the exception of pH: *Drymis* reaches a higher final value.

As regards the *permanent incubation* experiment: *Nothofagus* once more predominates. Soluble matter accumulated under anaerobiosis in all cases (Table 4), as compared with aerobiosis, the pH being low. The pH of the extracts increases in the order *Nothofagus* — *Lomatia* — *Drymis* and generally during incubations; it is lower in the anaerobic extracts and almost always higher in the second aqueous extract when lipids are removed. This indicates a chemical difference between the two extracts. Total reducing power, fulvic and humic fraction and soluble extract decreased during the experiment in a general sense. But the rate of change varied greatly, flushes of liberation and of degradation occurred during the course of the incubation.

With exception of *Drymis*, the reducing power of the water extract following removing of fats and waxes increased both in aerobiosis and anaerobiosis, as time of incubation was progressing; yet this was not the case with the weight of extracted soluble matter (Table 5).

Table 4

Level of soluble matter, in % of dry initial material, during incubation at 28°C (60 days)

Species	Days of incubation	Under aerobiosis						Under anaerobiosis					
		H_2O extract 1	pH	Ether extract	H_2O extract 2	pH	Total	H_2O extract 1	pH	Ether extract	H_2O extract 2	pH	Total
Notho-	10	17.0	4.0	5.8	3.9	4.5	26.7	16.7	4.1	5.8	3.4	4.6	25.9
fagus	20	13.5	3.5	7.0	3.7	4.1	24.2	16.8	3.4	7.6	2.8	4.2	27.2
	60	10.3	4.2	6.6	3.1	5.3	20.0	19.6	4.0	5.8	3.7	4.3	29.1
Loma-	10	9.3	4.8	4.8	2.6	5.1	16.7	11.2	4.4	4.9	2.4	4.5	18.5
tia	20	5.6	5.5	4.7	2.9	5.0	13.2	11.4	4.5	4.7	2.8	4.6	18.9
	60	4.3	5.8	4.4	2.1	5.4	10.8	9.4	4.8	5.0	2.9	5.1	17.3
Dry-	10	7.7	6.2	4.5	1.8	5.5	14.0	9.6	5.2	4.6	2.3	5.2	16.5
mis	20	4.4	6.6	5.8	2.2	5.8	12.4	6.6	5.0	5.2	1.6	5.2	13.4
	60	4.6	6.7	6.0	0.9	6.6	11.5	5.7	5.4	4.8	1.5	5.7	12.0

Table 5

Reducing power of soluble matter extracted from 100 g of dry material incubated during 60 days at 28°C in ml of 0.02 N thiosulphate

Species	Days of incubation	Under aerobiosis, aqueous extract					Under anaerobiosis, aqueous extract				
		1	2	Total	Fulvic acid 1	Humic acid 1	1	2	Total	Fulvic acid 1	Humic acid 1
Notho-	10	4723	381	5104	4231	493	5313	413	5726	4830	483
fagus	20	1663	530	2193	1452	121	1974	373	2347	1692	246
	60	1824	1178	3002	1600	226	2717	1193	3910	2358	246
Lomatia	10	723	458	1181	602	60	878	101	979	702	101
	20	452	167	619	422	30	420	134	554	350	70
	60	588	456	1044	495	45	1344	471	1815	1170	30
Drymis	10	755	555	1310	664	60	1208	673	1881	1026	120
	20	930	389	1319	739	151	1232	294	1526	1161	105
	60	652	178	830	483	181	788	229	1017	633	70

The fraction corresponding to the ether extract did not vary greatly, it was not affected by the presence or absence of O_2, nor by acidity.

The CO_2-evolution in presence of O_2 is parallel to the rate of release of soluble matter and reducing power. *Nothofagus*, and in a lower measure, *Lomatia* remains are subjected to mineralizing processes which are very active during the first stage of the incubation. The rate of mineralization reached after 60 days is quite high for all three species (Table 6).

The values obtained when the samples are reincubated are somewhat lower, but follow the same trend. It is seen clearly that the level of respiration depends on the continual release of energetic material from the substrate.

Under N_2-atmosphere, CO_2-evolution is low, though an ephemeral flush during the first ten days takes place with *Nothofagus* and *Lomatia*. There

Table 6

Cumulative CO_2-evolution, during 60 days at 28°C

Species	Aerobiotic		Aer., reincub.		Anaerobiotic		Anaer., reincub.	
	gC% OM	R_m	gC% OM	R_m	gC% OM	R_m	gC% OM	R_m
Nothofagus	1.819	3.13	1.418	2.44	0.408	0.70	0.469	0.81
Lomatia	1.464	2.52	1.047	1.80	0.387	0.67	0.327	0.56
Drymis	1.224	2.11	0.867	1.49	0.291	0.50	0.371	0.64

R_m = rate of mineralization of organic material (OM).

is little difference between values obtained under permanent incubation and reincubation, the latter being somewhat higher in the case of *Nothofagus* and *Drymis*. This proves that the accumulated soluble material is not only little mineralized but exerts some inhibition.

In general, the rate of mineralization reached after 60 days under permanent aerobiotic incubation is reduced to about 1/3 of this value under reincubation, whereas under anaerobic conditions it is rather higher.

Within each group of relations, the differences due to species are less than those due to influence of type of metabolism or treatment (Table 7).

Table 7

Relation of activity (respiration) according to mode of incubation (Ae = aerobic, An = anaerobic, R = reincubated)

Species	Ae/An	Ae_R/An_R	Ae/Ae_R	An/An_R
Nothofagus	4.46	3.02	1.28	0.88
Lomatia	3.78	3.20	1.40	1.18
Drymis	4.20	2.34	1.41	0.78

DISCUSSION

The process of humification in the litter, as a subsystem in which the sorptive properties of allophane are not yet intervening, is considered as a step of energy-flow in the ecosystem and as a link between epigean biomass and soil subsystem. In this sense the water-soluble matter, as representing the input through lixiviation of fallen, decomposing leaves, has been studied in detail. The considerable change which takes place in the composition of this aqueous extract (dry weight, pH, reducing power) under controlled conditions of incubation, indicates the possible patterns of change in nature.

Permanent incubation, under constant, optimal conditions of temperature and aeration, or anaerobiosis, represents a relatively closed system as functioning during periods where rainfall does not lead to percolation. Metabolites accumulated under absence of O_2 exert their limiting effect, but also under aerobiotic conditions some released substrates may lower the activity of degradation. Thus, a potential activity in relation to total substrate, including the complex, dynamical interaction between microbes and released metabolites is evaluated.

Successive reincubation yields insight into the actual activity in the field when material is percolated by rain or saturated with water. The amount of energy coming from the remains per unit weight and time, is evaluated along the course of degradation.

This procedure also allows for establishing a potential activity, in vitro, per period of incubation, in relation to the total possible release of water-soluble matter from accumulated remains.

The activating or inhibitory effect exerted by the accumulated metabolites is reduced, being eliminated periodically. Reincubations in a range of time allow for extrapolating a zero time, at which takes place a constant flow of energy from the plant remains, related to the flow through this relatively open system, with less or without interference from accumulated metabolites.

CO_2-evolution is generally lower in reincubation under aerobiosis, as compared with permanent incubating because some energetical substrates are removed before being degraded, but much more CO_2 is released than previsible if the available carbon would be released at once, i.e. come from the extract. Evidence is given that energy is constantly released even after prolonged incubation: a steep slope corresponding to the mineralization of accumulated soluble material precedes a flattened part of the curve, equivalent to the phase of degradation of material progressively released from the plant remains. This pattern characterizes individually the species we examined.

In some cases, CO_2-release is higher in reincubation than under permanent incubation, due to the periodical elimination of inhibitory metabolites, mainly organic acids as occurring with *Nothofagus* and *Drymis* under anaerobiosis.

CONCLUSIONS

In dead leaves which had accumulated under natural conditions up to periods of one month, but which had not yet come into contact with soil, humification is already under way. The humic acid fraction is still low as compared with fulvic acids.

Under laboratory conditions of constant temperature (28°C) and humidity (field capacity or saturation), part of the acid soluble fraction is metabolized during the first stage of the incubation, giving rise to a strong CO_2-evolution, both under aerobiosis and anaerobiosis.

Comparison of permanent incubation with successive reincubation shows that the accumulated metabolites lower very markedly the rate of metabolism under anaerobiosis, not only due to low pH.

It is interesting to note that the reducing power of matter extractable with water after extraction of lipids is quite high in *Lomatia* and *Drymis* leaves, as compared with the first aqueous extract. It appears that per unit dry weight of extract, more reducing power is obtained with the second; this holds particularly for *Nothofagus* where the reducing power of the second extract increases suddenly during the course of incubation. Under natural conditions this occluded matter seems to constitute a stock whose availability is delayed because physical accessibility is restricted by waxes and essential oils which are degraded very slowly.

The pattern of metabolism of *Nothofagus dombeyi* leaves is noteworthy: the high content of soluble energetical matter, its strong acidity, the high reducing power of extracts of dead leaves which are falling continuously during the cycle of seasons, are linked with the key role played by the acid-soluble fraction of humified matter of this species in the ñadi ecosystem. Moreover it is clearly seen how the initial chemical composition of plant remains projects onto further steps of metabolism.

Humification proceeds according to species, after an initial flush of mineralization as indicated by CO_2-evolution. Thus, the three dominant species in ñadi vegetation *(Nothofagus dombeyi, Lomatia hirsuta, Drymis winteri)* with an estimated production of dry leaves of resp. 2.0—1.3—2.1 T/ha/year yield a material which, in absence of the allophane complex, humifies and mineralizes well.

The energy-flow in ñadi ecosystem is slowed down by the adsorption of humified organic matter and formation of complexes with the mineral colloid.

SUMMARY

Ñadis are hydromorphic soils derived from volcanic ash in the alluvial plains of southern Chile; they may tentatively be classified as aeric andaquepts (duric). Though a high level of phreatic water saturates them during a large period in winter and despite of their high acidity, their potential biological activity is quite remarkable. The natural vegetation is a climatic virgin woodland of high biomass and low production; it is characterized by an association of relictual subtropical and antarctic species, mainly evergreen, and by a marked stratification of the forest canopy.

Under natural conditions the plant remains are humified without delay and completely, but mineralization is impeded, which leads to accumulation of humified organic matter in the form of complexes with aluminium and strongly adsorbed by the allophane, the anionic exchange capacity of the mineral colloid being thus maintained near saturation.

Factorial analysis under laboratory conditions have shown that the slowness of the process of mineralization is not due to deficiency of the relevant physiological groups of microbes, but pertains to a most efficient mechanism of ecosystem regulation.

Humification and mineralization govern, by the way of feed-back mechanisms, the entropy level of the system. In order to yield insight into energy flow within the ñadi ecosystem we compared the course of experimental organic matter metabolism, under controlled conditions of incubation both aerobiotic and anaerobiotic, of lead leaves and root system, alone or mixed with organic topsoil or mineral subsoil (allophane) of some of the more representative species of the Ñadi.

CO_2-evolution was chosen as a measure of global activity of the microflora, fulvic and humic acids were characterized by their reducing power and total analysis of the plant remains showed periodically the changes accompanying the synthesis of humus.

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AMMONIFICATION OF ALFALFA RESIDUES IN FLOODED AND MODERATELY WETTED SOILS

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In the irrigated soils of Central Asia and Kazakhstan, alfalfa root residues are known to be the main source of organic matter. Here cotton, sugar beet, rice and other crops are cultivated. When the technical cultures are grown the soil periodically is wetted by irrigation at the time when the rice soils are under water for 3—4 months.

Therefore, we considered it would be interesting to study the microbiological decomposition of alfalfa root residues in flooded and in moderately wet soils. Kovalev (1938) studying the effect of different conditions of irrigation: insufficient — 25%, optimal — 60% and surplus — 100% established that in the process of alfalfa decomposition in sierozem the decreasing of nitrogenous and carbonic components of plant residues occur at an unequal rate. More nitrogen in residues is retained under less moisture, but under surplus wetting nitrogen is released quickly in the form of ammonia.

In our comparative investigations the rate of deamination of plant proteins in flooded and moderately irrigated soils (45 per cent of the total moisture) 250 mg finely cut alfalfa roots were placed into the soil in polyethylene bags. Then periodically (in 10, 15, 30, 45 days) the amount of the nitrogen formed in decaying organic matter was determined. In analogous redox conditions, but under evenly distributed roots in all the soil mass we studied the content of ammonium nitrogen, which was determined colorimetrically with Nessler's reagent. The alfalfa roots were put in vessels with meadow-boggy soils from Kizil-Orda district, where the rice is cultivated (Ilyaletdinov 1970).

Under moderate wetting the rate of ammonification of plant protein, which is expressed by the decreased content of nitrogen in the roots was found to be considerably lower, than in flooded conditions.

In flooded soil intensive deamination of alfalfa proteins took place. For a month and a half following this period, the amount of nitrogen in plants declined from 246 to 155 mg/100 g of dry roots under moderate wetting and to 43 mg when flooded, that is to 37 and 82.6 per cent respectively (Fig. 1).

The ammonium released from alfalfa was found in the soil in the form of ion. When flooded more ammonium invariably was accumulated, than under moderate wetting. Thus, under flooding, better conditions for deaminating of plant proteins and soil organic matter by spontaneous microflora and for accumulating ammonium nitrogen were created (Fig. 2).

The decomposition of crop residues in the soil was closely connected with the activity of soil microflora and enzymes. The changes in number of

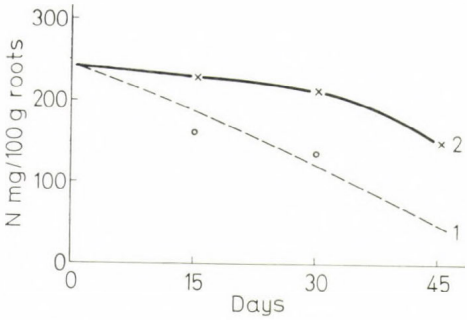

Fig. 1. The change of nitrogen content in the alfalfa roots in different conditions: flooding (a) and moderate wetting (2)

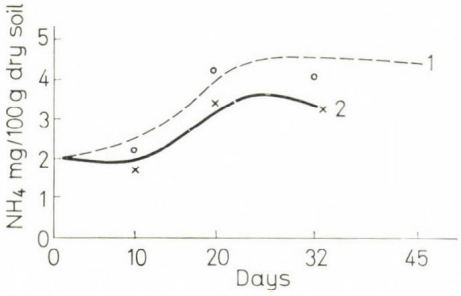

Fig. 2. The change of ammonium content in the soil by flooding (1) and moderate wetting (2)

ammonifying bacteria growing on meat-and-peptone agar, and microorganisms assimilating inorganic nitrogen (Czapek medium) during alfalfa roots decomposition in flooded and moderately wetted soil were observed. Hence it could be concluded that flooding suppressed activity of these groups of microorganisms (Table 1).

In moderately wet soil the number of microorganisms increased continually. Thus, the number of ammonifiers in the 7-day incubation was 7.6 million in 1 g soil and on the 45th day was 21.5 million. Quantity of fungi and bacteria, growing on the Čzapek medium, increased from 8.6 million to 53.7 million in 1 g soil. As it appears from the above, there is no tight dependence between the increase in the number of microorganisms in the soil and the release of ammonium from crop residues.

Table 1

The change of number of microorganisms during alfalfa root decomposition in flooded and moderately wetted soil (million/1 g of dry soil)

	Incubation period, in days				
Soil	7	14	21	30	45
	Ammonifiers				
Flooded	4.2	3.7	1.3	2.1	1.4
Moderately wetted	7.6	10.8	14.0	17.2	21.5
	Fungi and bacteria assimilating inorganic nitrogen				
Flooded	3.6	2.8	1.7	2.3	1.8
Moderately wetted	8.6	12.3	16.5	35.4	53.7

We were interested in defining the effect of alfalfa roots on proteolytic activity in soil. In one variant of the experiment the soil was mixed with fresh-dried alfalfa roots, in the other, the soil was mixed with alfalfa roots autoclaved at 0.5 atm for 30 minutes. The soil without alfalfa roots served as the control. Toluene was used as antiseptic for suppressing the activity of soil microorganisms. For determining proteolytic activity of

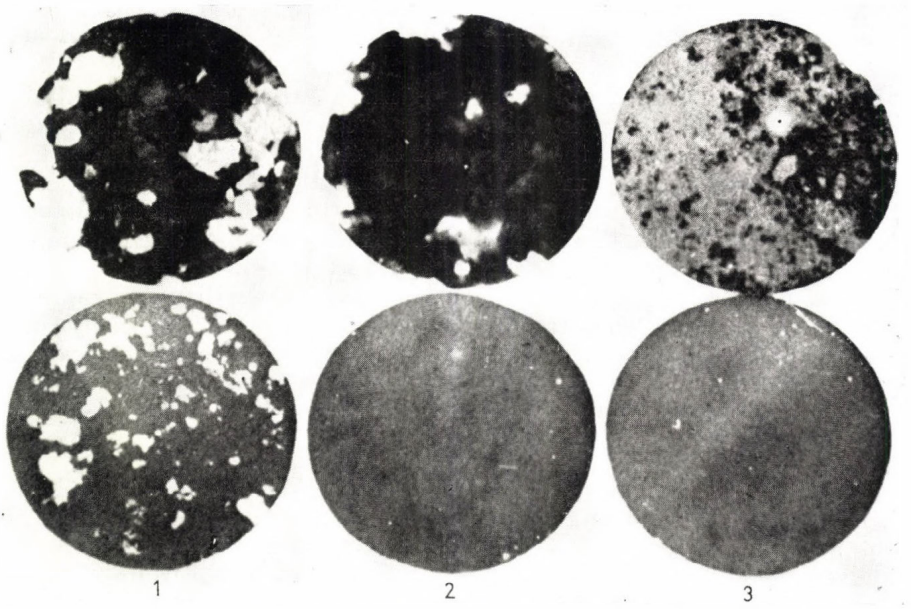

Fig. 3. The proteolytic activity of soil. 1. soil + fresh alfalfa roots; 2. soil + autoclaved alfalfa roots; 3 soil. Upper line — the soil without toluene; lower line — toluene is added to the soil

the samples of the soil they were distributed on the gelatin layer of photographic paper in Petri dish, wetted and incubated at 30°C for 72 hours (Vostrov 1967).

Very high activity of proteases was observed in the soil with fresh alfalfa roots, lower activity in the variants with autoclaved alfalfa roots, and even lower in the soil without alfalfa. By addition of toluene to the soil (the lower line in Fig. 3) the decomposition of the gelatin layer of photographic paper took place only in the soils with freshly dried alfalfa roots. In the soil with autoclaved alfalfa, as in the soil without alfalfa the decomposition of gelatin did not occur.

Consequently, the alfalfa root residues which were not subjected to thermal treatment had protease activity and served as the source of enrichment of the soil with enzymes, thus increasing total biological activity. Inactivation of proteases with high temperature caused sharp decrease in proteolytic activity in variants with autoclaved roots. Toluene suppressed enzymatic activity of microorganisms and therefore with the addition of antiseptic to the substrate the activity of proteases, only crop residues were retained.

DISCUSSION

By studying the regularity of microbiological decomposition of alfalfa root residues in the soils with different degrees of wetting we found discrepant facts on first inspection. In flooded soils, on the one hand, alfalfa proteins radiently decay with the release of ammonium, on the other hand,

the rapid decrease of activity of proteolytic enzymes was observed. In the moderately wet soil, ammonium accumulates slightly but proteolytic enzymes are very active.

For interpretation of observed phenomena we consider it essential to point to a thesis of Harmsen (1964). He considers that the transformations of carbon and nitrogen in the soil are distinguished by the fact, that carbon is rapidly volatilized by microbiological oxidation in the atmosphere in the form of CO_2, while nitrogen becomes transformed repeatedly. In other words, nitrogen released from plant and animal protein as ammonium may immediately transform to protein of soil microorganisms.

Based on this hypothesis, we consider that in the flooded conditions ammonium released from crop residues is not fixed in the composition plasma of microorganisms in consequence of small numbers of microorganisms in such soils. In the moderately wet soils ammonium produced, is rapidly assimilated by numerous microorganisms.

SUMMARY

In flooded soil more ammonium nitrogen produced as a result of alfalfa root protein decomposition is accumulated than in moderately wet soil.

Under flooding the quantity of saprophytic microorganisms is rapidly decreased, while protease activity is reduced simultaneously. Under moderate wetting, relatively high numbers of microorganisms in the soil correspond to considerable activity of proteolytic enzymes in the soil. The reason for low ammonium content in the moderately wet soil may be its utilization by microorganisms, oxidation by nitrifiers and fixation by soil minerals.

In the soil with alfalfa root residues in the first week after addition of organic matter, the high proteolytic activity is observed, which is probably connected with activities of enzymes of plant tissues. The roots subjected to heat treatment (in autoclave) have no such activity. The suppression of soil microorganisms' activity by means of toluene delays the occurrence of activity of proteases in the soil.

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PROTEOLYSIS AND BIOLOGIC ADSORPTION OF NITROGEN AT LOW TEMPERATURE

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The microbiological processes occurring in soil are thought to be mainly due to the activity of mesophilic microflora. This is probably why the laboratory investigations on soil microorganisms are as a rule made at 25—35 °C, this temperature being optimal for mesophiles. In soils of the temperate zone, however, such thermal conditions are quite uncommon. In warm periods the soil temperature does not exceed 20 °C and in cold ones drops to about 0 °C or below, the latter lasting up to 6 months. Under such conditions the activity of mesophiles in the soil ceases and their work is taken over by the organisms capable of growth and metabolism at low temperature.

The occurrence of such organisms in different soils is reported by many authors (Waksman 1916, 1922; Lochhead 1926; Rahno 1960; Kononova 1963; Stokes and Redmond 1965; Stefaniak 1968). As it can be seen from their reports the populations of the soil bacteria able to grow under cold conditions go into thousands and even millions of cells in 1 g of soil. Little do we know, however, what physiological groups they include and what processes may occur under their influence in the soil exposed to long lasting temperature of 0 °C. On the whole, the bacteria are thought to be able to carry on different metabolic processes, but the low temperature limits their activity and these processes may almost cease or go very slowly.

In this paper an attempt has been made to find in the garden soil the occurrence of proteolytic and nitrate assimilating bacteria capable of growth at near 0 °C. Their reaction was also investigated when glucose (5g/kg) and inorganic nitrogen (NH_4NO_3 = 75 mgN/kg) were added to the soil, as compared to the reaction of the same groups of bacteria displaying growth at higher temperatures.

The garden soil was taken towards the end of winter and to maintain winter conditions, stored in a cold chamber at 0—2 °C. Samples of soil and those of the same soil enriched with C, N and C+N were inoculated on selective media and incubated at 2° and 20 °C.

In the period of the experiment (over 3 weeks) the number of proteolytic bacteria capable of growth at 2 °C amounted to 200 to 400 thousand cells in 1 g of control soil and that of nitrate assimilating bacteria varied between 3 and 4.5 million. This constituted about 50—80% and 30—50% of the respective groups of bacteria displaying growth at 20 °C, which would indicate that the low temperature bears a stronger limiting effect on growth than on the survival of the nitrate assimilating bacteria, whereas in the case of the proteolytic bacteria it seems to be the other way round.

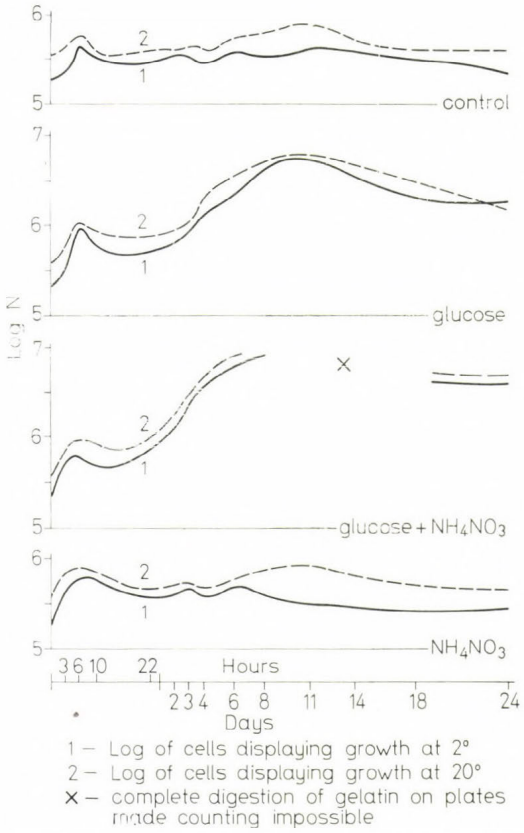

Fig. 1. Proteolytic bacteria in garden soil; stored at 0—2 °C with C, N and C + N added. N = number of cells referred to 1 g of soil, X = complete digestion of gelatin on plates made counting impossible. 1 = cells displaying growth at 2 °C, 2 = the same at 20 °C

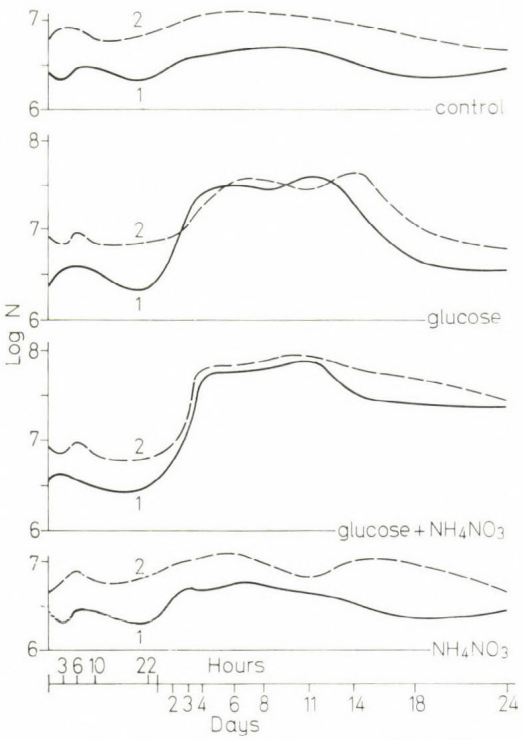

Fig. 2. Nitrate assimilating bacteria in garden soil; stored at 0—2 °C with C, N and C + N added. N = number of cells referred to 1 g of soil, 1 = cells displaying growth at 2 °C, 2 = the same at 20 °C

The reaction of both bacteria groups in question to glucose and NH_4NO_3 was the same at 2° and 20 °C, although its course was somewhat different in proteolytic bacteria and those assimilating nitrate. As it can be seen from the diagrams, the former reacted to the additions almost instantly and the reaction increased gradually (Fig. 1), whereas in the latter the lag phase lasted almost 24 hours and was followed by a rapid increase in their number (Fig. 2); they reached their maximum within 3 days and amounted to the same number at 2° and 20 °C.

Both groups demonstrated a very strong reaction to glucose or glucose and NH_4NO_3, the number of cells rising almost tenfold in each case. The state of unsettled biological equilibrium persisted much longer due to the addition of C and N combined than of glucose alone.

It was found that the inorganic nitrogen added alone to the soil does not affect its biological balance. The biological adsorption of nitrogen occurs

only when it is added simultaneously with organic substance. Only the proetolytic bacteria displayed a slight reaction to nitrogen in the first hours following its addition to the soil.

The results of investigations presented here as well as those obtained in similar investigations on arable soil (in print) show that the soil temperature near 0 °C does not inhibit the biological processes connected with nitrogen changes in soil. Although in the soil with settled biological balance the bacteria are less numerous than those able to grow at higher temperature, none the less, their number is a considerable one. No doubt they also have their by no means small share in the cycle of elements under the conditions of the temperatue which suppresses the growth of other bacteria. These bacteria seem to receive too little attention with regard to the role they play in the soil.

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ROLE OF MICROORGANISMS IN THE DECOMPOSITION OF MOSSES

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There are only a few microbiological works which deal with the decomposition of mosses. Studies which appeared on this subject refer to *Sphagnum* mainly (Poschenrieder and Beck 1958, Puffe, Grosse-Brauckmann 1963, Chastukhin 1967, Le Borgne et al. 1967). Other authors published data on the decomposition of mosses belonging to *Eubrya* (Waksman and Tenney 1928, Mikola 1954, Goetzke 1963, Kilbertus 1968b).

Experiments carried out so far demonstrated that the fragments of *Pseudoscleropodium purum* Hedw. Fleisch could be found for a long time (5—10 years) on the soil surface (Kilbertus 1968a). The speed of the decomposition in semi-natural conditions is expressed in the per cent of the yearly weight loss; in the case of the part of the living mosses was 27% and parts which are dying 30% and in dead parts 38% (Kilbertus 1968b).

Data of Mikola (1954) reported that the yearly weight loss of the green part of *Pleurozium schreberii* was 25.5% of the *Hylocomium splendens* 21.7% and the yearly weight loss of *Rhytidiadelphus triquetrus* 16.3%. These are in agreement with the results obtained by us.

These data prove that this litter is decomposed much more slowly than the litter of the deciduous trees. The decomposition of mosses differs from the former in the degree of decomposition on *Bryophila* fragments which can be demonstrated in every phase of the year, while in the deciduous trees a layering may be observed.

EXPERIMENTAL PART

In the course of direct microscopic investigations of the green parts of *Pseudoscleropodium purum*, we established that on these, besides a brown coloured *Mycelia Sterilia* fungus, two other fungi could be found sometimes. One of these was *Chrysosporium pannorum*, the other belongs to the *Oidiodendron echinulatum* species.

On the parts which were brown or dead, we could observe two fungi belonging to the *Basidiomycetes*.

The basidial fungi cultivated from mycorrhiza of forest pine *(Pinus silvestris)* as well as the mycelia of different moulds do not cause weight loss in the parts of *Ps. purum* which had been previously sterilized. Their role under natural conditions could not be clarified. Electronmicroscopic investigations (Kilbertus et al. 1970) confirmed that the basidiomycetes do not attack the cell wall of mosses.

The mycelia of the above mentioned two basidiomycetes disappear under "in vitro" conditions. It may be supposed that they cannot decompose the organic matter of the brown and dead moss. Under natural conditions, it may be expected that on the effect of favourable microclimate and due to the absence of concurrent species, they multiply on the surface of the moss. According to Chastukhin's (1967) data, the *Collybia dryophila* actively takes part in the decomposition of sphagnum.

Several fungi were cultivated from moss colonies which were studied by us on media using the method described in our earlier works (Kilbertus, 1968b, 1969). *Alternaria tenuis*, *Cladosporium herbarum* and *Epicoccum nigrum* were the most frequent species on green leaves. These were followed by *Mortierella ramanniana*, *Trichoderma viride*, *Penicillium spinulosum*, *Chrysosporium pannorum* and *Mucor hiemalis* species. The latter could be identified during the whole time of decomposition and they probably have an important role in mineralization.

The third group consisted of *Chaetomium indicum*, *Cephalosporium* sp. and *Oidiodendron rhodogenum* species.

Under natural conditions the *Pseudoscleropodium purum* occurs with *Brachypodium pinnatum* grass. On this grass many such fungi could be found which did not develop on moss but the mycoflora of *Ps. purum* also occurred on the grass.

The water extract prepared from the *Ps. purum* did not affect the growth of the investigated microscopic fungi but the extract inhibited the fungi not occurring in mosses but on grass. Bacteria are also very sensitive to the extracts because their numbers decreased intensively when its concentration is high.

Chemical analysis proved that the cellulose material of the mosses could not be utilized by the well-known cellulolytic fungi. Čzapek already described in 1899 that there is a material present in the cell wall of Sphagnum which inhibits the microorganisms. For this reason in the case of mosses, the dye reaction of cellulose could be detected, only after they were treated with 1% NaOH or alkalic hyperchlorite solution (Bünning 1927).

The resistance to decomposition of cellulose of *Ps. purum* could be explained by the presence of Sphagnol or the special composition of the cellulose of mosses. Since our chromatographic investigation demonstrated a large amount of glucose, the special feature of the cellulose of mosses is not likely. Sphagnol extracted with sodium hydroxide from mosses already inhibited the cellulolytic activity of *Trichoderma viride* in diluted solution.

According to our investigations, this inhibition effect is due to the presence of two phenolic compounds, especially the p-hydroxy benzoic acid and p-cumaric acid belong to the material which can be extracted with sodium hydroxide. Supposedly they do not take part only in the inhibition of cellulose decomposition but the mechanical defense of Sphagnol could play an important role here.

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ROLE OF THE SOIL MICROSCOPIC FUNGI IN THE DECOMPOSITION OF PLANT RESIDUES

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In the literature one can find many works connected with the microbiological decomposition of plant roots remaining in the soil; these influenced the organic matter balance of the soil to a great extent which has a slight connection with its fertility (Kononova 1951, Tyurin 1937).

The microscopic fungi have a very important role in the soil formation processes and generally in the cycle of matters. Because of their extraordinary wide-spectra ferment system they have a basic importance in the mineralization of the organic material introduced into the soil.

Kostychev (1886) firstly pointed out, that the dark coloured pigment materials formed in the course of decomposition of plant residues were carried out by microscopic fungi. These organisms have intensive ammonification properties. Mishustin and Pushkinskaya (1942), Geltser (1940) and other authors emphasized the important role of microscopic fungi in the soil structure and humus formation.

Many authors drew attention to the fact that in the first stage of the decomposition of plant residues introduced into the soil the microscopic fungi have the main role. Their activity declines with the progress of assimilation of the organic materials which can be utilized more easily. Other soil fungi do not take part in the first phase of the mineralization (Mishustin and Pushkinskaya 1942, Mishustin and Timofeeva 1944, Chastukhin 1952).

EXPERIMENTAL PART

On the basis of the above, our aim was to study the dynamics of soil fungi. The soil samples needed for the experiment were collected partly from the dark chestnut soil of the north part of the Kazakh Soviet Socialistic Republic, uncultivated virgin soil and sample taken from spring wheat sowing. Soil samples also were taken from irrigated desert gray meadow soil sown with lucerne in the South part of Kazakhstan. The qualitative and quantitative ratios of the more important fungal groups were determined in these investigated soils. The chestnut carbonaceous soils occupy enormously large territories in the north part of the Republic where the climate is very poor in moisture. These soils which are mainly uncultivated have a potential fertility. The weight of root residues is 20—24 ton, their humus content 300—400 ton, their nitrogen content 20 ton, their phosphorus acid content 15—20 ton/hectare calculated for the upper 1 m soil level (Matyshuk et al. 1961). The most organic residues, the humus, as well

Table 1

The quantity of plant residues, humus and fungi in dark chestnut soils

Cultivation phase	Depth in cm	Plant residues ton/h	Humus, %	Fungi 1000/g soil
Virgin soil	0—7	8.9	4.74	11.3
	7—14	4.0	3.74	6.5
	14—22	2.2	3.02	3.4
	22—30	1.6	2.74	2.8
Harrowed (with disc harrow) soil	0—7	8.5	4.71	30.6
	7—14	2.9	3.85	13.0
	14—22	2.2	3.08	8.2
	22—30	1.4	2.75	2.4
Ploughed soil	0—7	6.1	4.24	21.9
	7—14	3.2	4.08	18.3
	14—22	2.0	4.02	16.2
	22—30	1.6	3.0	2.7

as the majority of the fungal flora are concentrated in the upper so-called grassy level (Table 1).

As it was mentioned the plant of the cultivated soil was spring wheat at the time of sampling. Its root system is very resistant to the mineralization activity of microscopic fungi. During the five investigation periods about 40—60% of the root mass was decomposed in the virgin soil uncultivated. Grossbard's (1969) investigation proved also that the decomposition of root system of cereals takes place relatively slowly.

Most humus in the virgin soil could be found similarly to the plant residues in the highest part of soil. In the layer harrowed, the amount of humus decreases. In the course of ploughing, the distribution of humus becomes widespread especially in the part turned up it decreased while in the under part it rose. But the loss of humus is not significant because of the extracts of the carbonaceous dark chesnut soils. The change resulting from the climatic conditions of the arid steppe zone — according to the literature data — needs 15—20 years more time (Orlovsky 1935, Matyshuk et al. 1961).

The majority of the microscopic fungi are zymogenic microorganisms occurring in the investigated soils and mineralizing the fresh plant materials (Winogradsky 1952). It can be seen from the table that the maximal number of microscopic fungi as can be demonstrated in the highest layers of the soil and going to the deeper layer decreased strongly. Yatchevsky (1933), Naumov (1933), Kursanov (1940), Mishustin (1951) as well as other authors received the same results. It is interesting that with the depth of the layers not only the number of fungi decreased but the length of fungal hypha too. It is very likely that the soil biochemical activity is decreased too with this.

On the effect of the cultivation of the virgin soils the number of fungi increases significantly. Especially a large amount of fungi could be demonstrated with 0—7 cm upper part on the effect of harrowing. The fungi oc-

curred in deep ploughed soils, since plant materials get into the deeper soil layers too with ploughing.

The change in the amount of microscopic fungi is determined by the moisture conditions too (Table 2). This is typical mainly for the upper level of the soil which is more prone to drying. The moistening of soil increases the number of fungi rapidly.

Table 2

The change of the number of fungi taken as a function of soil moisture in dark chestnut soil

Cultivation phase	Depth of cultivation in cm	May		June		July		August	
		I	II	I	II	I	II	I	II
Virgin soil	0— 7	27.6	15.8	11.1	5.8	17.9	12.8	6.1	—
	7—14	27.7	10.6	18.2	4.6	11.0	4.4	17.5	—
	14—22	25.8	4.4	17.7	3.2	12.5	2.8	14.4	—
	22—30	25.6	3.0	19.1	2.6	16.1	2.8	14.2	—
Horrowed (with disc harrow) soil	0— 7	38.1	42.0	16.5	21.6	27.8	38.0	6.1	21.0
	7—14	40.7	11.6	23.8	15.6	15.6	10.2	12.2	15.2
	14—22	31.4	5.2	23.2	11.8	15.9	3.4	13.1	12.6
	22—30	28.9	3.4	22.9	2.2	16.7	1.8	14.6	2.4
Ploughed soil	0— 7	27.7	30.2	13.5	14.6	25.5	22.6	7.1	15.4
	7—14	34.9	26.0	26.6	15.8	28.2	17.6	15.5	13.8
	14—22	36.3	26.8	26.6	12.0	20.2	14.8	16.1	11.2
	22—30	32.2	3.8	27.5	3.2	20.4	2.0	16.9	1.8

I Soil moisture in % of the maximal water capacity.
II Number of fungi, 1000/g soil.

The qualitative analysis of the microfungi proves that the fungal species of flora of the investigated dark chesnut soil is not variable. Mainly that species occurred here, which do not have demands as regards the moisture and nutrient material sources. Their majority occurred in the upper part of the ploughed soil but *Penicillium* species could be found in all soil levels during the whole vegetation period. Species belonging to the *Mucor*

Table 3

The quantity of plant residues and microscopic fungi in the desert gray meadow soils

Time of sampling	Depth in cm	Plant residues ton/h	Number of fungi 1000/g soil
May	0—20	5.9	10.5
	20—40	2.1	5.7
June	0—20	3.9	11.9
	20—40	1.6	6.8
October	0—20	1.1	9.7
	20—40	0.8	4.8

Table 4

The number of fungi in desert gray

Year	Depth in cm	Penicillium			Aspergillus		
		I	II	III	I	II	III
1964	0—10	—	4.2	2.3	—	3.4	3.0
	10—20	—	3.6	3.0	—	2.0	1.4
	20—30	—	3.0	2.4	—	1.6	0.8
	30—40	—	1.9	1.0	—	0.8	0.3
1965	0—10	1.8	2.0	3.6	1.6	2.0	4.0
	10—20	1.8	2.2	3.0	1.6	1.6	2.0
	20—30	1.2	1.2	2.0	0.4	0.8	1.2
	30—40	1.6	1.6	1.8	0.8	0.8	1.0

genus which can survive the dry conditions were concentrated in the upper soil level, but *Trichoderma* species were dominant in the deeper soil level, rather often but not in such large numbers. *Aspergillus* occurred and more rarely the species belonging to the *Alternaria*, *Fusarium* and other genera. The most intensive cellulolytic kind are the representatives of *Myrothecium*, *Chaetomium*, and *Globisporus* genera. The weakly cellulose-decomposing species belong to the *Trichoderma* and *Fusarium* genera.

In the next part of our investigations the fungal flora of the irrigated desert gray meadow soils were examined. Lucerne cultivation which is one of the basic ways of increasing soil fertility was conducted on this soil type. The weight of the lucerne-root mass counted in 1 m depth was 19.6 ton/hectar; from this 13.38 ton was in the upper 26 cm level, the layer between 26—60 cm had 5.47 ton lucerne root mass. The decomposition of the lucerne and the different grass-roots started after the spring ploughing (Table 3).

As it can be seen from the data of the table, 36% of the plant residues were mineralized in the 0—20 cm layer after ploughing in two months time (in June). In the 20—40 cm layer the mineralized plant residues were at the same time 20%. These demonstrate that the residues of lucerne are mineralized more rapidly than the dead root system of the spring wheat. One of the main reasons for this is that there is no unfavourable effect due to the lack of moisture as in the chestnut soil because these are irrigated areas.

According to the data of Table 4 the "total" number of the fungi is much less in this soil type than in the above-mentioned one. It is very likely that the spore-forming processes slow down under favourable moisture conditions but this does not exclude the important role of the vegetative mycelia in the transformation of the plant residues.

As the data show the number of fungi was higher before ploughing of lucerne than in summer time. However it is visible that *Penicillium* and *Aspergillus* species are dominant firstly, but species belonging to the *Fusarium* genus are represented in considerable number too. The number of *Mucor* and *Trichoderma* species is not significant.

meadow soils (1000/g soil)

Fusarium			Trichoderma			Mucor		
I	II	III	I	II	III	I	II	III
—	2.6	1.2	—	2.0	0.5	—	1.4	1.6
—	2.0	1.4	—	2.0	0.8	—	1.4	1.0
—	1.0	0.6	—	0.6	0.6	—	0.8	0.8
—	0.2	0.2	—	0.2	0.2	—	0.2	0.2
1.0	0.6	0.2	3.0	3.8	1.6	3.8	4.4	2.0
1.0	0.4	0.2	2.4	3.0	1.2	3.0	3.8	1.6
0.4	0.2	0.2	2.4	2.8	1.0	3.0	3.6	1.6
0.2	0.2	0.2	0.6	0.8	0.3	0.8	1.4	0.4

Note: I = spring, II = summer, III = autumn.

After ploughing the species spectrum of microscopic fungi changes. The decomposing plant materials supply the microorganisms with considerable amounts of nitrogen and easily available carbohydrate. In this phase the number of fungi belonging to *Mucor* and *Trichoderma* genera surpassed the number of *Aspergillus* and *Fusarium* species. Generally the number of fungi is higher except that of *Fusarium* species, but the ratio related to each other is not changed significantly. In autumn when the amount of easily available plant materials increases and the amount of plant fractions decomposes more slowly, the number of fungi belonging to the *Mucor*, *Trichoderma* and *Fusarium* significantly decreases, then the number of representatives of *Penicillium* and *Aspergillus* genera increases.

SUMMARY

In the transformation of the plant materials getting into the soil, the microscopic fungi — having a wide-spectrum ferment system — play a great role.

The residues of the lucerne grown and ploughed down in the desert-gray meadow soil mineralized much more quickly than the root residues of spring wheat in the dark chestnut of dry steppe zone. The soil fungi changed qualitatively and quantitatively in the course of the mineralization of the plant residues.

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DECOMPOSITION OF CELLULOSE AND FIXATION OF NITROGEN IN THE SOILS OF VARIOUS TYPES

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Insufficient elucidation of the role of the free-living nitrogen fixers in the nitrogen balance of the soil can be accounted for by the difficulties which are involved in determination of small increases of nitrogen in soils according to the Kjeldahl procedure as well as by the fact that in the soil there can simultaneously occur the processes (elution, denitrification) which lead to the losses of soil nitrogen.

Therefore, the use of $^{15}N_2$ for the assay of the soil for nitrogen fixation is of great interest (Delwiche and Wijler 1956; Rice and Paul 1964; Brouzes et al. 1969). The use of ^{15}N permits detection of fixation even in very small amounts of the molecular nitrogen repesenting about 0.02 per cent of the initial content of nitrogen in the soil.

Investigations were carried out on the nitrogen-fixing activity of the soil samples collected from different climatic zones.

The experiments were carried out in vacuum dessicators filled with a gas mixture containing 20% O_2, 30% $^{15}N_2$ and 50% argon. The soil samples were placed in a thin layer of not more than 1.5—2 cm in thickness into the small cylindrical glass cups or Petri dishes. The soil was saturated with water.

Investigation of nitrogen fixation was conducted with or without (the control) addition of exogenous organic carbon-containing substances like cellulose or glucose. Each treatment was in triplicate. Released CO_2 was trapped by KOH. Addition of oxygen into the desiccator replacing the oxygen utilized by the soil was performed 2—3 times per week. Incubation was carried out in the dark during 4—6 weeks at 26—28 °C and the ^{15}N level was afterwards determined in the soil samples on mass spectrometer.

Utilization of cellulose by the soil was determined with anthrone reagent after treatment of the samples by 70% H_2SO_4 for 16 hrs in cold.

Table 1 shows data characteristic of the nitrogen fixing activity of the soil under natural conditions where the nitrogen fixation occurs on account of the organic substances available in the soil itself. Nitrogen-fixing activity of the soil can be associated with the organic pool of the soil whose presence might be indicated by the occurrence of carbohydrates. Thus, the total carbohydrate in the sodic-podzolic and chestnut soils as determined with anthrone reagent was not more than 1.7—2.5 mg per g soil while in chernozem and meadow bog soils it amounted to 6—10 mg per 1 g soil.

Introduction of organic substances (cellulose, glucose) into the soil exerted a marked stimulating effect on nitrogen fixation in the studied

Table 1

Fixation of nitrogen by various soils under natural conditions (without addition of exogenous organic substances)

Soils investigated	Excess of atom % ^{15}N in soil	Excess of atom % ^{15}N samples	N_2 fixed mg/kg soil	Fixation of nitrogen calculated per 1 hectare arable layer, kg/hectare
Sod-podzolic arable soils, Moscow region	0	—0.025	0—0.21	0—0.63
Gray forest virgin soils, Tula region	0.00		0.0	0.0
River basin soils, Tula region	0.06		0.78	2.35
Chernozem virgin soils	0.02	—0.06	1.0—1.4	3—4.2
Chernozem arable soils	0.0	—0.01	1.0	3.0
Chestnut arable soils	0.0	—0.01	0.5	1.5
Meadow-bog soils, Moscow region	0.20		5.5	16.5
Meadow-bog soils under rice, I*	0.160		7.74	23.2
Tashkent region II* *	0.465		22.5	67.5

Note: Duration of incubation — 4 weeks.
N_2 containing 95% ^{15}N was used.
* Incubation in cups.
** Incubation in Petri dishes with a thin layer of soil.

Table 2

Productivity of nitrogen fixation during decomposition of cellulose in various soils

Soils investigated	Treatments	Excess of atom% ^{15}N in the soil	Fixed N_2 mg per 1 kg soil	Fixed N_2 mg per 1 g cellulose
1	2	3	4	5
Sod-podzolic, Moscow region	+2% cellulose	2.32	31.0	1.55
Grey forest, virgin, Tula region	+1% cellulose	0.60	15.25	1.52
River basin, Tula region	+1% cellulose	1.45	19.0	1.9
Chernozem, Kursk region, virgin soil	+2% cellulose	0.80	40.6	4.06
Mountain chernozem, Alma-Ata region, virgin soil	+2% cellulose	3.04	76.8	3.84
Chestnut virgin soil, Alma-Ata region	+2% cellulose	7.3	91.5	4.58
Dark-brown virgin soil, Zaporoshje region	+1% cellulose	0.82	23.2	2.32
Meadow solonchak, virgin land, Zaporoshje region	+1% cellulose	2.75	45.5	4.55
Meadow-bog soil under rice, Tashkent region	+2% cellulose	4.77	230.0	11.5

Note: The samples were incubated with nitrogen containing 95% atom % ^{15}N.

soils. Most interesting was the investigation of the productivity of nitrogen fixation on addition of cellulose to the soil which is the main constituent of plant remnants entering the soil.

In Table 2 data are presented on the productivity of the nitrogen fixation in the soil due to cellulose decomposition.

Productivity of the nitrogen fixation in different soils varied markedly. Maximum productivity of nitrogen fixation (up to 11.5 mg N per g cellulose) was found in the paddy soils. Nitrogen fixing productivity in chernozem and chestnut soils varied over the range of 2.5—4.5 mg nitrogen per g cellulose. A lower level (an average of about 1.5 mg N per g cellulose) was found in sod-podzolic and grey forest soils.

It should be noted that low nitrogen-fixing activity found in sod-podzolic soils might often be due to the absence of active cellulose decomposing microflora capable of forming a productive symbiosis with nitrogen fixers. As evidenced from Table 3 exogenous cellulose practically was not decomposed in the soil and nitrogen fixation did not occur, yet addition of glucose and lactate induced active nitrogen fixation.

In grey forest soils, exogenous cellulose was utilized rather effectively and low nitrogen fixing productivity was apparently due to a low activity of microorganisms present. Exogenous cellulose was readily decomposed in chestnut soils and especially in those under rice. Most of the soils investigated, yielded microbe associations containing cellulose-decomposing and nitrogen-fixing bacteria and were capable of active nitrogen-fixation on account of decomposition of cellulose.

Table 3

Nitrogen fixation by the sod-podzolic soil upon addition of various sources of carbon

Source of carbon	Excess of atom %	N_2 fixed mg/kg soil	Productivity of nitrogen fixation mgN_2/g of carbon source	Initial amount of carbohydrates in the soil, mg/g		
				In the beginning of the experiment	at the end of the experiment	uptake of cellulose
The control without additions	0.00	0.0	0.0	2.9	2.3	0.6
+0.5% cellulose	0.00	0.0	0.0	7.9	6.7	1.2
+0.5% glucose	0.55	24.4	4.88	—	—	—
+0.5% lactate	0.87	39.5	7.90	—	—	—

Note: Duration of incubation — 4 weeks.
Nitrogen containing 25 atom % ^{15}N was used.

The species pattern of these complexes varies grossly. In microbial association isolated from rice field soils, decomposition of cellulose is operated by obligately anaerobic bacteria while microbial complexes isolated from meadow grey and chernozem soils are inhabited mainly by cellulose-decomposing myxobacteria *(Cytophaga hutchinsonii)*. In associations investigated,

Azotobacter was found but occasionally and the major role was played by facultative symbiotrophic fixers belonging to mycobacteria.

The important role of these forms in nitrogen fixation by the soil is evidenced from our data by a marked increase in the numbers of this group of nitrogen fixers by addition of cellulose to the soil. These results are shown in Table 4.

Table 4

Changes in the number of facultative symbiotrophic nitrogen fixers by addition of 0.5% cellulose to the soil

Soil samples	The number of symbiotrophic nitrogen fixers (thou sand/gsoil)	
	Initial	After incubation with cellulose
River basin soil, Tula region	25.0	25,000.0
Chernozem, Kursk region	27.3	250.0
Ordinary chernozem, Donetsk region	13.4	13,000.0
Chestnut soil, Alma-Ata region	27.3	250.0
Meadow solonchak, Zaporozhje region	148.0	110,000.0

Note: The number of nitrogen fixers was determined by the method described earlier (Kalininskaya 1967).

The findings support the conclusion that the activity of free-living nitrogen fixers can play an important role in restitution of the soil nitrogen reserves. The presence of organic substances, entering the soil together with plant residues, as well as a propitious hydrothermic regime are indispensable for active nitrogen fixation by the soil.

SUMMARY

The activity of the free-living N-fixing bacteria was studied in a soil environment with ^{15}N isotope method. A large amount of nitrogen was fixed in the (loamy) meadow soils containing much organic material, where the N-amount fixed fluctuated between 6—7 mg/kg soil.

In the chernozem soils the fixed-N was 0.5—2 mg/kg soil; in the sod-podzolic soils and in grey forest soils no significant nitrogen fixation was observed without the addition of organic materials.

The addition of cellulose and glucose to the soil increased significantly the nitrogen fixation.

The highest produtivity of nitrogen fixation (8—11.5 mg N/g of cellulose) added was observed in soils under paddy cultivation. In chernozem and chesnut soils productivity of nitrogen fixation fluctuated from 2.5 to 4.5 mg N fixed/g of cellulose utilized. A lesser significant productivity of nitroden fixation was noticed in sod-podzolic and grey forest soils.

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EFFECT OF FERTILIZATION ON THE ACTIVITY OF CELLULOLYTIC BACTERIA AND BREAKDOWN OF CELLULOSE IN THE SOIL

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Data of the literature have indicated an intensification of the cellulolytic process in the soil as a result of the application of mineral fertilizers (Kadota 1956; Unger 1960; Zakharov and Atamyuk 1960).

We proposed to study the effect of increasing mineral fertilizer doses on the development of cellulolytic bacteria and breakdown of cellulose in the soil with a view to investigating the possibility of controlling the mineralization processes of the organic residues in the soil.

For this purpose fertilization field experiments conducted in 1969 on two soil types were used leached chernozem-smolnitza (experimental field at the Bajourishte village) and leached cinnamonic forest soil (experimental field at the Grigorevo village). The experiments were carried out with maize. The following variants of increasing fertilizing doses were tested: $N_{60}P_{40}K_{40}$; $N_{120}P_{80}K_{80}$ and $N_{240}P_{160}K_{160}$ after application of 5 tons. of farmyard manure.

The number of cellulolytic bacteria was assessed periodically during vegetation of plants over 7 months with 30 days' intervals. Kadota's (1956) agar-containing mineral medium as modified by Zakharov (1960) was used for counting of cellulolytic bacteria. Assessment of the breakdown intensity of cellulose in the soil was done after Unger (1960). The change in the available nutrient (NO_3^- and P_2O_5) content was also observed during vegetation in fertilized and nonfertilized plots by means of colorrimetry.

Results (Fig. 1) show that the mineral fertilizers which were applied, increased the accumulation of nitrates and mobile phosphorus forms in the soil. Increased doses of applied mineral fertilizers augmented the content of nitrates and mobile phosphorus forms. NPK variants accumulate more nitrates in the arable layer (0—20 cm) and the mobile phosphorus is accumulated equally in the arable layer and subsurface layer. There are differences in nitrate accumulation between both soils studied, in that more nitrates are shown to accumulate with the cinnamonic forest soil. This can be attributed to the more favourable conditions under which the nitrification process proceeds.

Data on the effect of increasing mineral fertilizer doses on the development of cellulolytic bacteria in the soil are given in Fig. 2. They show a different numbers of cellulolytic bacteria not only between both soils investigated but between the different fertilizing variants as well.

Comparing the number of these bacteria in chernozem smolnitza and in leached cinnamonic forest soils it was found to be higher in the latter type.

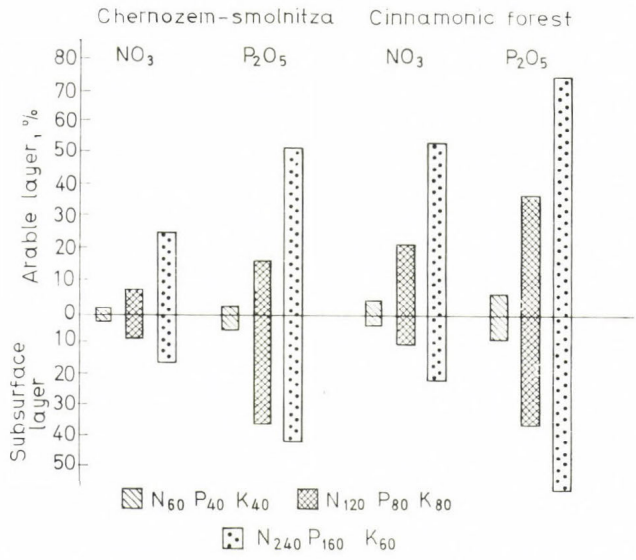


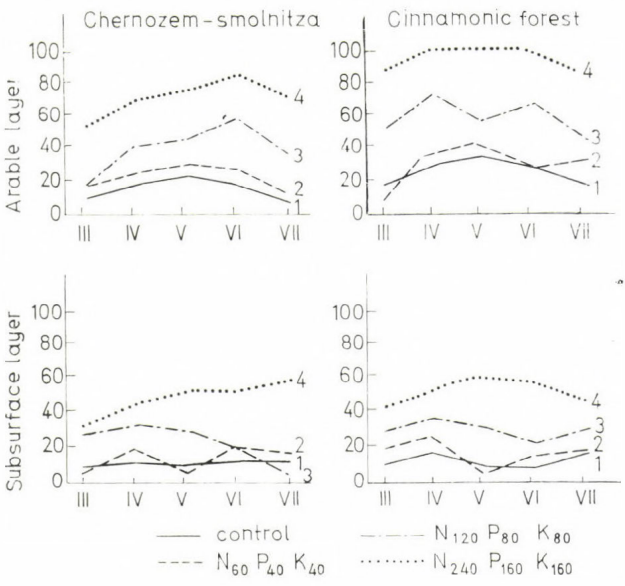
Fig. 1. Increase of the mobile nutrient forms as compared to the control

This was observed during the whole period of the study owing to the optimal nitrate accumulation in this soil.

The development of cellulolytic bacteria was stimulated by the higher doses of mineral fertilizers as shown by the data of the Figures. In our experiments, e.g. lower mineral fertilizer doses ($N_{60}P_{40}K_{40}$) did cause a small if any change in development of the cellulolytic microorganisms in both soil types. In this variant the number of cellulolytic bacteria is similar to that of the control. Higher applications (doses of $N_{120}P_{80}K_{80}$ and $N_{240}P_{160}K_{160}$) pointed to a substantial number increase of the bacteria analysed. The increased number of cellulolytic bacteria was higher when applying a higher mineral fertilizer dose.

The activity of the cellulolytic process is reflected in Fig. 3 which gives data on the changes of cellulolytic effect of the soil. When a certain quantity of cellulose remains in the soil over a period of 7 months the highest percentage of cellulose decomposition was observed with the variants of higher fertilization doses. The lower mineral fertilizer doses had a slight effect on the cellulolytic activity both in the chernozem-smolnitza and cinnamonic forest soils.

Fig. 2. Effect of applying mineral fertilizers on the activity of cellulolytic bacteria (% of crumbs attained)



The cellulolytic activity was much higher in the tilth top of the soil as compared to the subsurface layer of both soils studied which correlated with the higher nitrate quantity in the former.

The great importance of the mobile nutrients for enhancing the mineralization of the organic matter in the soil was confirmed by the data on the changes occurring in the number of cellulolytic bacteria and the cellulose-decomposing activity of the soil. According to the results obtained, there is a definite correlation between the content of mobile nutrients in the soil and the intensive development of cellulolytic bacteria and the cellulose-decomposing activity of the soil. The higher content of mobile nitrogen (NO_3^-) and phosphorus (P_2O_5) forms is correlated with the higher numbers of cellulolytic bacteria and higher percentage of cellulose broken down in the soil.

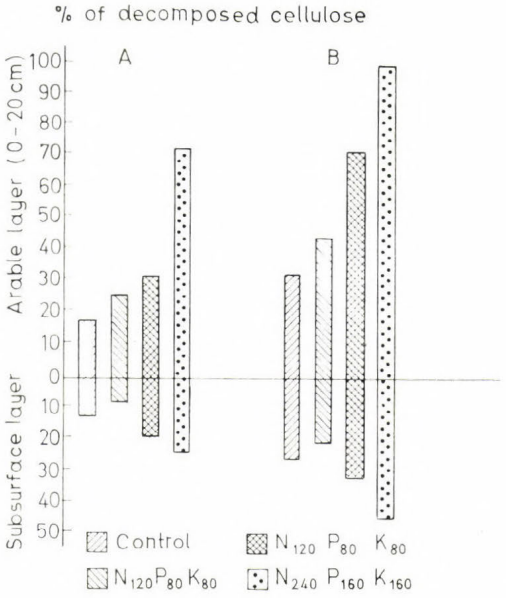


Fig. 3. Change of the cellulose-decomposing activity of the soil as affected by mineral fertilization. (A) chernozem-smolnica, (B) cinnamonic forest

Applying higher mineral fertilizer doses intensified the microbiological processes, related to the mineralization of the organic matter, thus favouring the creation of more favourable conditions for plant growth.

According to the maize yield results, the higher mineral fertilizer doses resulted a higher yield increase. This is closely correlated with the more intensive development of the cellulolytic bacteria and increased cellulose-decomposing activity.

The following conclusions may be drawn:

1. Higher mineral fertilizer doses stimulated the number of cellulolytic bacteria and cellulose-decomposing activity of the chernozem smolnitza and cinnamonic forest soils.
2. There is a certain correlation between the content of mobile soil nutrient and the intensive development of the cellulolytic bacteria and the cellulose-decomposing activity of the soil.
3. The higher cellulolytic soil activity is correlated with higher maize yields suggesting that the cellulose-decomposing capacity index reflects correctly the changes in the soil following fertilization.

SUMMARY

Data are presented on the effect of the application of various doses of mineral fertilizers, viz.: $N_{60}P_{40}K_{40}$; $N_{120}P_{80}K_{80}$ and $N_{240}P_{160}K_{160}$ on the development of cellulolytic bacteria and intensity of cellulose decomposition.

Two soil types were studied: leached chernozem-smolnitza and leached cinnamonic forest soil.

Results allow for the following inferences:

1. Mineral fertilizers applied in higher doses have a stimulating effect on the number of cellulolytic bacteria and cellulose-decomposing activity of the chernozem-smolnitza and cinnamonic forest soils. This stimulative effect is more characteristic in the cinnamonic forest soil.
2. There is a definite correlation between the content of mobile nutrients in the soil and the intensive development of the cellulolytic bacteria and the cellulose-decomposing activity of the soil.
3. The higher cellulose-decomposing activity of the soil is correlated with higher maize yields which is a good reason for supposing that the cellulose-decomposing activity index correctly reflects soil changes at fertilization.

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TRANSFORMATION OF CARBON-CONTAINING SUBSTANCES IN SOILS OF CENTRAL SIBERIA AS CHARACTERIZED BY THE CELLULASE ACTIVITY

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In the course of recent years two independent trends have branched out in modern soil microbiology: 1) investigation of microbe varieties in the soil and of regularities governing their ecologo-geographic distribution, 2) investigation of biochemical processes going on in the soil under the influence of microorganisms.

The destruction of plant residues falling into the soil happened by enzymes. As cellulase is one of the dominant substances that get into the soil, the study of its transformation processes caused by microorganisms is of undoubtedly great interest and as for the cellulose disintegration intensity, it can be measured by the soil cellulolytic activity.

The author has investigated the cellulase activity in the soils of Central Siberia and in the microorganism cultures isolated from these soils. The test in question may serve as an index of mineralization intensity in plant substances in the soil (Kislitsina 1966, 1968). The objects of investigation were soil samples from the mountain and plain-grown taiga as well as from the steppes.

The cellulase activity was measured by the colorimetric method, with the use of anthrone, from the quantity of glucose (the final product of hydrolysis), which is formed as a result of cellulose mineralization (Kislitsina 1966).

The intensity rate of the cellulose mineralization in different soils of Central Siberia reflects the general direction of the mobilization processes in the soil and may testify to the level of the soils being provided with available forms of carbon (Kozlov and Kislitsina 1967).

As was formerly established, the cellulase activity increases from North to South and from the mountain-grown taiga soils to the steppe soils (Kislitsina 1966).

Most likely the excess moisture and the shortage of heat in the mountainous regions, the long-term seasonal soil freezing and the phenomena of cryogeneity create conditions hindering the physiological activity of microflora, which results reduction of cellulase activity and, hence, a repression of organic substance disintegration in the soils of Siberia.

There are conditions promoting the development of microflora in general and the cellulose-decomposing microflora in particular, we can observe edophotope concentration by exoenzymes and growth of exoenzymic activity causing intensification of organic substance mineralization processes. It is characteristic to a higher degree of the southern taiga soils and to a lesser degree of the steppe soils.

A change in the cellulase activity in the course of time depending on oscillation of hydrothermal factors of the medium has been established. Thus two maxima in the enzymic activity — the late spring maximum and the autumn maximum — can be observed in the steppe soils of Zabaikalye. They coincide with the quantity of cellulose-decomposing microorganisms which are chief producers of cellulases in the soil. A sharp drop of enzymic activity and a decrease in the quantity of microorganisms which are responsible for the cellulose decomposition in the soil are observed in these soils in summer.

The above phenomenon in the conditions of the Zabaikalye steppes can be first of all explained by the moisture shortage in the soil which hinders the development of microflora and consequently leads to an insignificant organic disintegration in summer.

The cellulose-decomposing microorganisms which are capable of producing exoenzymes (cellulase) and which participate in the transformation of organic substances in the steppe soils include: *Penicillium, Chaetomium globosum, Chaetomium spirale, Aspergillus fumigatus, Aspergillus niger, Alternaria tenuis, Trichoderma lignorum, Dematium sp. Fusarium sporotrichioides, Rhizopus nigricans;* the Actinomycetes including *Actinomyces roseus, Actinomyces griseus,* the bacteria including *Sporocytophaga myxococcoides.* Participating in disintegration of organic substances and formation of cellulases in the soils of the southern taiga are cellulose-decomposing mushrooms: *Dematium, Trichothecium, Chaetomium, Alternaria, Mucor, Trichoderma, Penicillium, Stemphylium;* the dominant variety of actinomycetes being *Actinomyces griseus;* the bacteria including *Sorangium.*

Investigation of the cellulase activity in the soils of different landscapes has brought out a general tendency of all soils subject of investigation a decrease in the activity with the depth. This phenomenon is associated with a sharp decrease in the quantity of vegetation and root residues across the soil profile with accumulation of mobile forms of nitrogen (chiefly in the upper soil levels) which is of great importance for the activity of cellulase — for the decomposing organisms.

Investigation of litter and its differentiation into fractions (moss + A^0, A^{II}, A^{III} — for all fractions) brought out the following regularity: the highest activity of cellulases is shown by the fraction "moss + A^{0II}. Most likely it is here that the organisms process the greatest quantity of organic substance in the form of various carbohydrates including glucose and an accumulation of this substance in the layer "moss + A_0" is going on. Therefore the highest cellulase activity is observed here. This activity may serve as an index of organic substance disintegration activity in the soil under the influence of microorganisms producing cellulases (Table 1).

Investigation of interconnection of edificatory varieties of plants in the taiga zone, the soil microflora and the cellulase activity in the soils showed that the main role in disintegration of organic substances in the southern taiga soils belongs to mushrooms and bacteria and, to a lesser degree, to actinomycete.

Table 2 shows that the cellulase activity in the rhizospheric soil is the highest under raspberry and the lowest under currant. The proportion of cellulose-decomposing mushrooms, bacteria and actinomycetes varies depending on the investigated plants. The cellulase activity is well correlated

Table 1

Organic substance disintegration intensity in the litter as exemplified by cellulase activity

Description of fraction	Cellulase activity in milli-units, ml/min		
	litter fractions Moss + A_0	A_0''	A_0'''
Spruce-fir fine grass	0.36	0.21	0.25
Spruce-fir diverse grass	0.21	0.18	0.14
Fir-horsetail-sedge	0.20	0.19	0.12
Spruce-cedar-fir fine grass	0.21	0.16	0.14
Fir-spruce with cedar, diverse grass	0.8	0.06	0.01
Spruce-fir-woodreed	0.24	0.10	0.09

Table 2

The quantity of cellulose-decomposing microorganisms in the rhizosphere of dominant varieties of plants in the southern taiga and their cellulase activity

Plant	Number of microorganisms in % per 1 g of soil			Cellulase activity in milli-units ml/min
	mushrooms	bacteria	actinomycetes	
Currant	2.04	10.20	87.72	0.03
Raspberry	42.84	68.34	8.26	0.15
Sedge	6.12	13.26	9.18	0.07
Fir	4.08	43.86	16.22	0.06
Willow	3.11	6.12	60.18	0.04

with the total quantity of cellulose-decomposing mushrooms both in the steppe soils, the taiga soils and in litter.

The material obtained in the course of investigation is undoubtedly indicative of the fact that an essential role in the organic substance transformation is played by microorganisms and their enzymes. The example of cellulase activity shows the direction and intensity of mineralization of carbon-containing organic substances that belong to the plant kingdom.

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LIGNOLYTIC ACTIVITY IN SOILS

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It is highly probable that the lignified materials embedded in the soil undergo degradation processes which are like those already known in the corresponding literature. These are:

1) The brown rot and soft rot, during which cellulose is decomposed while lignin is turning slowly into dark products.

2) The white rot in which all wood components are equally destroyed, lignin giving rise to biologically active phenolic monomers.

The distribution of both kinds of rot in the soils are at present almost unknown and we propose here the use of a new technique which may afford some information about these processes.

METHOD

In glass-jars (250 ml) 3 g leached sawdust, a piece of beech twig 4 cm long, a glass fibre bag containing 8 g leached sawdust were placed. The latter was prepared by autoclaving (1 hour) a mixture of beech sawdust

Table 1

Main characteristics of experimental soils and plant cover

No	Soil type	Parent rock	pH	Plant cover
Tx	Podzol (Alios)	Sandstone	3.4	*Pinus silvestris*, *Calluna vulgaris*
FSM	Podzolic soils (Pseudogley)	Loam	3.6	*Quercus sessiliflora* *Calluna vulgaris*
FSC	Podzolic soils (Pseudogley)	Loam	3.6	*Calluna vulgaris*
BFN	Rendzina	Terra fusca + Colluv.	7.9	*Fagus silvatica*
BFS (near BFN)	Brown forest soils, leached	Terra fusca	4.3 to 5.2	*Fagus silvatica* *Fraxinus excelsior*
NSC	Brown forest soils, leached	Terra fusca		*Fagus silvatica* *Carpinus betulus*
NSE (near NSC)	Brown forest soils,	Terra fusca		*Picea abies*
Mp	Brown mediterranean soil	Lime Colluvium	8.0	*Quercus ilex* *Hedera helix*

and water (1/10 w/v), filtering and washing off with cold distilled water until the filtrate become colourless. The glass jars were then sterilized using ethylene oxide.

After elimination of the volatile toxicant about 125 ml. of soil previously freed from organic debris (roots, leaves) were placed beneath the sawdust bag. The latter was cut open, the sawdust moistened with 15 ml water and moulded into a cake whose surface was easily observable through the jar cover.

The soil was saturated to its water holding capacity. At frequent intervals the initial weight was restored by adding some distilled water. At the same time the colour of the sawdust and presence of fructifications were noted.

8 forest soils were used most of which are located in Eastern France, the others in the vicinity of Paris (FSM, FSC) or Montpellier (MP) (Table 1).

For each soil 20 samples of the upper layer (A_0, A_1 horizons = 0—5 cm deep) and 20 samples of A_1, C, A_2 or B_1 horizons (depth 15—20 cm) were studied.

RESULTS

Fungal flora. During the incubation process the sawdust cakes were frequently examined and from time to time some pieces of sawdust were taken out and cultured on nutrient malt agar. Direct observation showed the presence of sterile mycelia, Dematiaceous fungi *(Phragmosporeae)* and discolouration of the cake associated usually with clamp bearing mycelia. On the culture plates grew common fungi such as *Trichoderma*, *Penicillium*, *Cephalosporium*, *Chaetomium*. The isolation in pure culture of the species observed on the cakes was often difficult and sometimes impossible.

After 18 months' incubation the cakes were taken out and the beech twigs picked from the soil, whose remnants were washed away. Finally the twigs were split lengthwise and incubated on moistened sand for direct observation. On the other hand small pieces of wood were taken away and subcultured.

The results indicate the rarity of dematiaceous fungi, and the frequency of sterile mycelia, *Xylaria polymorpha* and white rot fungi, the latter being often more common than on the corresponding cakes.

110 cakes out of 308 studied were more or less discoloured and their distribution is presented in Table 2.

Lignolytic activity. At the end of the experiment the cakes were dried at 105 °C and weighed. The percent loss of weight (W) gives a figure for the total biodegradative activity.

Then the lignin content was measured by a modified Klason method. From the residue, ash and "proteins" were deduced and the rest calculated as percent of initial lignin gives a figure (L) for the lignolytic activity.

Finally the relative lignolytic ability $\left(R = \frac{L}{W} \times 100\right)$ gives information on the rot-type prevailing in the soil under study. The average value for R is about 100 in the typical white rots induced by Basidiomycetes and 75 in the case of Ascomycetes.

Table 2

The number of the discoloured cakes according to soil type and group of fungi

Soil and depth, cm		Mull soils		Soil and depth, cm		Moor soils	
		Basidio-mycetes	Asco-mycetes			Basidio-mycetes	Asco-mycetes
MP	0— 5	1		FSC	0— 5	9	none
	15—20	1			15—20	4	none
BFS	0— 5	5	1	Tx	0— 5	9	none
	15—20	7	2		15—20	4	none
NSC	0— 5	2	4	FSM	0— 5	2	none
	15—20	3	4		15—20	2	none
BFN	0— 5	6	7				
	15—20	4	7				
NSE	0— 5	11	4				
	15—20	6	5				

The values of W and L show broad variations inside both horizons and for each soil and we attempted to divide their values into 5 classes. These are:

A = 0 to 9%, very weak loss,
B = 9 to 18%, weak loss,
C = 18 to 36%, middle average loss,
D = 36 to 60%, heavy loss,
E $\geq$ 60%, very heavy loss.

It became possible to represent the lignolytic ability of a soil by the relative frequency of the data in each class. The results as expressed in Table 3 are as follows:

1. *Total activity:* our data are in accordance with the current ideas.

In the upper layer, the activity of brown soils and rendzina are quite high (40 to 70% samples in D class); they are very weak in the podzolic soils, most of the samples belonging to classes B and C.

Exception must be made for a brown soil under spruce (NSE), in which C class is dominant and for a podzolic soil under *Calluna* (FSC) in which two maximal frequencies are found: one (50%) like that in the homologue soil under oak (FSM) for B and C classes and the second in E class (40%).

In deeper layer, biological activity is lower: the maximal frequencies are commonly found one class lower than in the corresponding upper layers. This effect is most evident in NSE under spruce (2 classes lower) and in the soil under *Calluna* (FSC) which comes closer to the next and very poor podzolic soil FSM.

2. *Lignolytic activity.* In each soil and horizon the variations of this activity are parallel to those of the total activity, although keeping one or two classes lower. In the upper layer, the dominating categories are A in two podzolic soils (Tx and FSM) and B or C elsewhere. The E class comprises a maximum of 15% of the individual data except under *Calluna* (FSC) where this figure rises to 30%. In the deeper layer the results are almost

Table 3

The numerical characteristics of the lignolytic activity of the investigated soils

Soil horizon and depth, cm		W/L					R			M
			0/9	9/18	18/36	36/60	60	I	II	III	
Mp	0— 5	W	0	0	15	55	30				53.5
		L	10	20	55	15	0	13	80	7	26.3
	15—20	W	0	30	35	25	10				33.3
		L	30	30	20	0	20	13	60	27	23.5
BFS	0— 5 (A_1)	W	0	5	10	70	15				49.3
		L	5	35	40	15	5	10	75	15	25.8
	15—20 (A_1C)	W	0	10	45	40	5				38.8
		L	35	30	30	0	5	20	65	15	18.6
NSC	0— 5 (A_1)	W	0	5	15	40	40				51.6
		L	15	10	40	10	15	35	40	25	30.7
	15—20 (A_2)	W	15	35	40	10	0				20.7
		L	60	25	10	5	0	45	30	25	10.0
BFN	0— 5 (A_1)	W	0	0	20	50	30				51.3
		L	20	30	25	10	15	35	45	20	26.3
	15—20 (A_1C)	W	0	0	40	55	5				40.3
		L	40	35	20	0	5	65	25	10	15.2
NSE	0— 5 (A_1)	W	5	0	55	20	20				37.9
		L	20	35	25	10	10	10	55	35	26.3
	15—20 (A_2)	W	55	5	30	10	0				17.8
		L	55	10	20	5	10	25	20	55	16.5
FSC	0— 5 (A_0/A_1)	W	5	25	25	5	40				41.8
		L	15	35	10	10	30	15	20	65	34.2
	15—20 (B_1)	W	70	10	20	0	0				15.6
		L	70	10	0	15	5	55	10	35	13.4
Tx	0— 5 (A_0/A_1)	W	10	20	50	15	5				25.8
		L	60	20	10	10	0	55	25	20	12.4
	15—20 (A_2)	W	25	50	25	0	0				14.8
		L	90	5	0	5	0	85	5	10	4.1
FSM	0— 5 (A_0/A_1)	W	15	40	40	5	0				19.5
		L	65	20	15	0	0	45	35	20	8.4
	15—20 (B_1)	W	85	5	5	0	5				8.7
		L	70	20	5	0	5	45	5	50	8.9

Notes: Columns W/L and R = number of samples in % of the total.
Column M = average loss,
W = loss of weight,
L = loss of lignin.

equally distributed amongst the A, B and C classes in the most active soils (Mp, BFS, BFN), class A being strongly dominant in the podzols (Tx, FSM).

3. *Relative lignolytic ability.* We recognize here only three different classes:

I from 0 to 30, II from 30 to 65 and III over 65. For 80 %, the noticeable white rots are to be placed in the III class, but in some samples where the fungus appears later or grows poorly, the R value varies from 30 to 50.

On the other hand, by some samples whose total activity is very low (A class) the values of R rise to 100 or more without any visible discolouration. This is the case for the deep layer of NSE soil where lignivore agents are frequent but also of FSC and FSM where they are less common.

We therefore admit that lignin is split more or less quickly in the soils where class III samples are numerous. This is the case for the A_1 horizon of NSE under Spruce and FSC under *Calluna* (35 and 65 % resp. in class III). Elsewhere in the upper layers of hardwood forest soils their frequency lowers to 15—20 % with a minimum of 7 % in the brown mediterranean soil (MP).

R is higher in deep horizons of poor soils (35 to 65 % in class III of FSM, FSE, NSE) but also in the highly active soil MP (27 %).

CONCLUSIONS

The suggested method allows the recognition of significant differences among the studied soils, regarding their total and lignivore activities. The first group comprises the leached brown soil under hardwoods and the rendzina, the activity of which is well-balanced and rather high in the deep layer. Close to it is the brown mediterranean soil, but the lignivore agents are very sparse: actually we do not know whether their absence is a general characteristic of this soil type or is related to the peculiar place studied.

The second group includes podzolic soils whose activities are weak even where lignivores are present. They are weaker in the deeper layer although lignin is sometimes as quickly destroyed as cellulose.

Both soils under Spruce and *Calluna* keep partially the same characteristics as those located in the vicinity but under hardwood. However they show a higher lignivore activity (average R = 70 in the upper layers; 100 in the deeper).

These conclusions are based on the study of only 8 stands for 3 years. It would be desirable if this method could be applied to a larger number of soils in order to set off general laws.

SUMMARY

A trapping method using water-extracted beech sawdust and beech twigs is proposed for the search for white-rot fungi in the soil. It permits also evaluation of the biological activity (W) as total loss of weight of sawdust, the lignolytic activity (L), as loss of lignin in the sawdust, and the relative ability (R) of a soil to degrade lignin, as figured by the quotient

$$R = \frac{L}{W} 100.$$

From the results obtained it appears that:

1. Every soil contains white-rot fungi but their abundance is variable. They belong usually to Basidiomycetes but *Xylaria* spp. are also important in certain soils. Beech twigs are more effective in trapping the latter and sawdust the former.

2. The individual values for R range respectively from 80 to 120 or from 25 to 40 in the presence or absence of white-rot fungi.

3. Amongst 10 forest soils (podzols, brown soils, rendzinas) the mean R values range from 40 to 70 but the significance of these differences seems generally low and will be studied later.

4. In most cases biological activity seems to be the main factor governing the lignolytic activity. Both values are less in podzol and higher in the brown forest soils under hardwood but not under spruce. The highest mean R-value was found in a podzolic soil under heather.

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STUDY OF THE FORMATION OF MELANOID PIGMENTS BY SOME ACTINOMYCETES AND MICROSCOPIC FUNGI

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There are many communications dealing with the biological and biochemical problems of pigment synthesis. According to the results of Mason (1956), Cromartie and Mason (1957), Bull and Faulkner (1964), Douglas and San-Clemente (1956), Kang and Felbeck (1965), Mencher and Heim (1962), Schanel (1967), Zenova (1965, 1968), among the soil microorganisms there are many such organisms which form and excrete dark coloured pigment materials in their surroundings. These materials are formed in the tyrosine-containing medium as a result of the quinone-type transformation or from the aromatic compounds synthethized by microorganisms without introduction of tyrosine. On the basis of these, the pigment-forming microorganisms are separated into two main groups in the literature.

In the first group are listed the microorganisms which synthesize melanin only in the protein- or tyrosine-containing medium. The organisms belonging to the second group form pigments in the synthetic medium without addition of tyrosine.

In the last case the microorganisms synthesize melanin as their own products having aromatic or quinone bases. According to Küster's findings (1963) some actinomycetes can form melanin from antibiotic products with quinone bases. Haider and co-workers (1965), Hurst (1967) supposed that the aromatic compounds formed in the course of metabolic processes as well as the aromatic lignin derivatives may be melanin sources under appropriate conditions.

The other basic condition of the synthesis of the melanin type materials: the presence of the phenoloxidase ferments. The main role in these processes is attributed to the tyrosine and laccase ferments which take part in the synthesis of materials having other quinone units, like e.g. humic acids too.

METHODS AND MATERIALS

Among the factors influencing the pigment synthesis the role of N-sources was investigated in detail. The physico-chemical properties of the pigment materials separated from the cultural medium were also studied. They were characterized by the determination of their UV absorption spectra as well as the separation of the pigment fractions chromatographically.

Pigment-formation of 5 ray and 5 microscopic fungi was studied in our experiments. The microorganisms were incubated in a liquid Czapek medium

modified in its composition. It contained 2% glycerol, C-sources and different N-sources according to the treatments. N-sources were tyrosine, yeast extract, glycine, $NaNO_3$ and in the fifth variant also $NaNO_3$ as N-source but in this case 0.02% syringaldehyde was added to the medium as a supplementary C-source. 500 ml Erlenmeyer flasks were used in which 100 ml nutrient materials were measured. The inoculations were carried out with the same amount of spore-suspension of fungi and ray fungi. The standing cultures (without shaking) were incubated at 28 °C for 4 months. At the end of the incubation the liquid was separated from the mycelin by filtration.

The pigment material in the culture liquid was precipitated with HCl — according to the method referred to Küster (1963) — after it was separated from the medium by centrifuging and it was washed with distilled water in the centrifuge tubes. The precipitation was extracted with n-butanol and the fraction soluble in n-butanol was marked as melanine material which was used for further investigations. The phenoloxidase was demonstrated with the plate-test method. The microorganisms were incubated in the above-mentioned medium, containing 2% agar-agar in this case. The tyrosine and laccase were evaluated on the basis of reactions formed with specific substrates.

The intensity of melanin formation was evaluated by the measurement of the optical density of the culture liquid. The measurements were made with spectrophotometer at 540 nm wavelength. The pigment material was determined with UV spectrophotometer at 240—360 nm. The separation of melanin fractions was carried out by chromatographic method, using Whatman No. 1 paper. The solvent system was n-butanol:acetic acid:water 2:1:1.

RESULTS AND DISCUSSION

On the basis of the experimental data the melanin production was the most intensive in the medium containing yeast extract and tyrosine. In the presence of these two N-sources the condition of the intensive development of mycelia was given, which stimulated the synthesis of the phenoloxidase ferments. But the oxidizable phenol-compounds were available almost unrestrictedly too.

In the medium containing glycine, only five ray fungi and three microscopic fungi formed melanin intensively. The melanin production of the L-6 marked *Fusarium* and the Ksz-14 *Penicillium* strains was considerably smaller. The rate of growth was satisfactory in this case too, but supposingly these fungi form aromatic compounds only to a lesser extent. In the presence of $NaNO_3$ as N-source, a smaller amount of melanin formed than in the medium containing glycine. The addition of syringaldehyde into the medium increased the melanin production. This indicates that the syringaldehyde added to the medium interacting with phenoloxidase becomes melanin. The melanin-type transformation of the aromatic lignin derivatives according to Haider's (1965) and Mason's (1955) publications takes place with phenoloxidase ferments in the presence of amino acid or protein-metabolic products. The amino acids or protein hydroly-

sates interact with the oxidizable phenolic materials and as a result of this interaction nitro- and dinitro-phenyl compounds are formed. From the mentioned compounds melanin or a humin-like product may be formed by means of oxidation, condensation and polymerizations.

It appeared from the data of the spectrophotometric measurements that the absorption maximum of the melanin type pigments of the investigated ray and microscopic fungi is in the 268 nm—280 nm region. The absorption curves show a well-expressed, characteristic peak in the band of the absorption maximum. In the former the smaller and less expressed second peak was observable in the pigment spectrum of the L-5 marked *Penicillium* and 1—56 marked *Streptomyces*, in the 290—300 nm region, but only in the case when glycine or yeast extract was the N-source. In the light spectrum of the pigment of 4—25 marked *Streptomyces* strain two absorption maxima were noticeable, but likewise only in case of glycine or yeast extract N-sources. The peaks were found at 270 and 279 nm. The treatments cause a smaller displacement of the absorption maxima which is represented on the spectrum taken from the melanins of other microorganisms.

Figures 1 and 2 demonstrate the UV absorption curves of the melanin products received from the culture medium of the P-511 marked *Penicillium*,

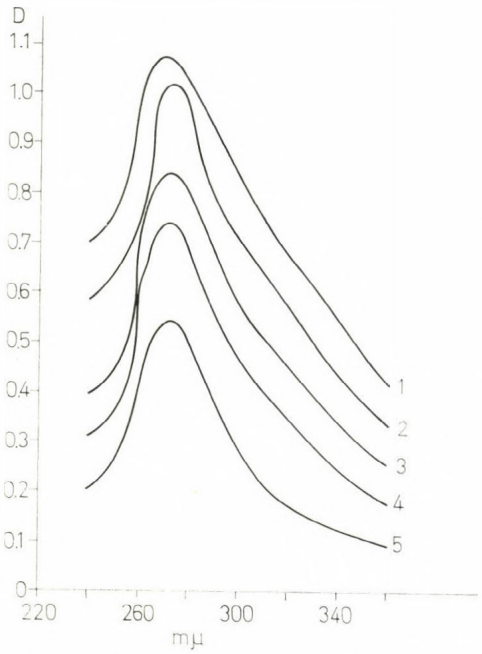

Fig. 1. The UV absorption of the pigments of *Penicillium sp. P. funiculum* series (No. P-511) incubated in the presence of different N-sources. 1 = Yeast extract, 2 = Tyrosine, 3 = Glycine, 4 = $NaNO_3$ + syringaldehyde, 5 = $NaNO_3$

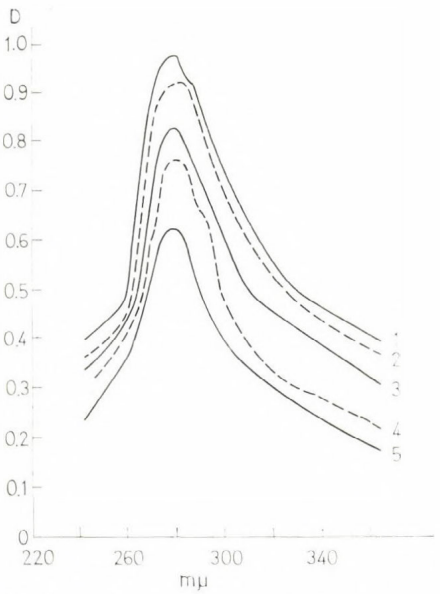

Fig. 2. The UV absorption curves of the pigments of the *Streptomyces sp.* series *Chartreusis* (No. 2—40) incubated in the presence of different N-sources. 1=Glycine, 2 = Yeast extract, 3 = Tyrosine, 4 = $NaNO_3$ + syringaldehyde, 5 = $NaNO_3$

Table 1

The formation of melanin-type materials and the R_f-values of the melanin fractions on the medium containing different N-sources

	Laboratory marking of the investigated micro-organisms	N-source	R_f-values of the melanin fraction						
			Brown	Yellowish brown	Red	Yellow	Brownish red	Pink	Yellow
Streptomyces	S—9	Tyrosine	0.0	0.07	0.77	—	—	—	—
		Yeast extract	0.0	0.17	—	—	0.56	—	—
		Glycine	0.0	0.22	0.77	—	0.56	—	0.94
	M—60	Tyrosine	0.0	0.07	—		› 40	—	—
		Yeast extract	0.0	0.14	0.78	0.87	-	—	—
		Glycine	0.0	0.01	—	—	0.40	—	—
	1—56	Tyrosine	0.0	0.07	—	—	—	—	—
		Yeast extract	0.0	0.17	0.79	0.85	—	—	—
		Glycine	0.0	0.20	—	—	0.56	—	0.93
	2—40	Tyrosine	0.0	0.09	—	—	0.36	—	—
		Yeast extract	0.0	0.14	—	0.85	—	—	—
		Glycine	0.0	0.1	—	0.83	—	—	0.93
	4—15	Tyrosine	0.0	0.07	0.76	—	—	—	—
		Yeast extract	0.0	—	—	—	0.55	—	—
		Glycine	0.0	0.05	—	0.87	—	—	—
Microscopic fungi	L—1	Tyrosine	0.0	0.1	—	0.87	0.57	—	—
		Yeast extract	0.0	—	—	0.87	0.56	—	0.93
		Glycine	0.0	0.32	—	0.85	0.56	—	0.93
	L—5	Tyrosine	0.0	0.07	—	—	—	—	—
		Yeast extract	0.12	0.26	—	0.85	0.56	—	—
		Glycine	0.11	0.25	0.70	—	—	—	0.92
	Ksz—14	Tyrosine	0.0	0.06	—	—	0.43	—	—
		Yeast extract	0.07	0.11	—	—	0.55	—	0.95
		Glycine	0.11	0.35	—	0.87	0.52	—	0.98
	P—511	Tyrosine	0.0	0.06	—	—	0.36	—	—
		Yeast extract	0.07	0.25	—	0.87	0.57	—	0.95
		Glycine	0.11	0.30	—	0.84	0.56	—	0.93
	L—6	Tyrosine	0.0	0.05	—	—	—	—	0.94
		Yeast extract	—	0.0	0.70	—	—	0.68	0.90
		Glycine	—	0.1	0.70	—	—	0.67	0.90

and 2—40 marked *Streptomyces* strains which were incubated on media containing different N-sources.

The composition of the melanin-type products of the microorganisms were investigated by means of chromatographic method. In Table 1 the R_f values of the different coloured fractions are shown which are separable in the applied n-butanol: acetic acid: water solvent system.

The pigments of the microorganisms growth on a medium containing tyrosine as N-source could be separated into two or three fractions, especially dark brown, yellowish-brown, red or brownish-red fractions. Yellow fraction was not formed with the exception of *Aspergillus candidus* (L-1). The melanin product of the microbes was separated into four or more fractions. Besides the brown, yellowish-brown and brownish-red fractions, a yellow fraction was found in every case, sometimes in two separated spots. The studied microorganisms synthesized lactase-type polyphenoloxidase; six of them produced tyrosinase too. Some correlation could be observed between melanin synthesis and phenoloxidase activity. The lactase and tyrosinase formation of the microorganisms on the media containing different N-sources is shown in Table 2.

Table 2

Phenoloxidase production of a few microscopic and ray fungi on medium containing different sources of nitrogen

Name of the investigated microorganisms	Tyrosine	Yeast extract	Glycine	$NaNO_3$	Tyrosine	Yeast extract	Glycine	$NaNO_3$
	Laccase				Tyrosinase			
Streptomyces flavovirens (S—9)	—	+	+	—	+	+	+	—
Streptomyces antibioticus (M—60)	+	+	+	+	—	+	—	—
Str. sp. *series Chartreusis* (1—56)	+	+	+	+	—	—	—	—
Str. sp. *series Chartreusis* (2—40)	+	+	+	+	+	+	+	+
Str. sp. *series Venezuelae* (4—15)	+	+	+	+	—	—	+	+
Aspergilus candidus (L—1)	+	+	+	—	+	—	+	+
Penicillium verruculosum (L—5)	+	+	+	+	+	+	+	—
Penicillium sp. *series P. purpurogenum* (K—sz—14)	+	+	+	+	—	—	—	—
Penicillium sp. *series P. funiculosum* (P—511)	+	+	+	+	—	—	—	—
Fusarium avenaceum var. herbarum (L—6)	+	+	+	+	+	+	+	+

The pigment of the investigated microorganisms could be characterized as melanin. This is proved by the data obtained from the UV absorption measurements, by the absorption properties of the synthetic DOPA-melanin or the dihydroxy-indole as well as by the natural melanin of animal origin. According to Stein (1955) the UV-absorption maximum of the DOPA-melanin can be found at 270 nm, the animal melanins at 275 nm. The absorption maximum of 5—6 dehydroxy-indole was detected according to Cromartie and Mason (1955) at 270 and 300 nm wavelength.

SUMMARY

The dark coloured materials formed by the investigated ray- and microscopic fungi have melanin character. The absorption maxima are in the 268—280 nm region; the fractions are separable chromatographically: the fraction composition depends to a great extent on the quality of N-source.

A correlation could be seen between the melanin formation and phenoloxidase activity.

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RELATIONSHIP BETWEEN THE DECOMPOSITION OF GLUCOSE BY SOIL MICROORGANISMS AND THE GROWTH CURVE OF *E. COLI* AS INFLUENCED BY CLAY MINERALS

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It has been observed since many years that the resistance of soil organic matter to biological transformation rises with the increasing content of clay minerals. Enhanced biological activity of autotrophic and heterotrophic soil microorganisms, the higher amounts of soil bacteria and the higher rate of their growth, and the lesser amounts of soil fungi were determined in the presence of a higher amount of clay minerals in soil (Zvjagintzev 1959, Stotzky 1964, 1966, Stotzky and Rem 1966, Sørensen 1967, Estermann and McLaren 1959, Macura and Pavel 1959). The effect of clay minerals on soil dynamics is very complex. The determination of the total amount of soil clay minerals and their individual types, the study of their effect on the enzymatic and microbial activity have become a still more important branch of soil fertility research.

This paper deals with the effect of two types of clay minerals, bentonite and kaolinite on the mineralization of glucose in a mineral nutrient solution and on the rate of growth of *Escherichia coli* cultivated in the same media.

MATERIALS AND METHODS

0.2 percent glucose dissolved in a mineral substance containing nutrient solution, was employed as the basic medium. The solution contained all the necessary biogenous elements in appropriate concentrations (Nováková 1968). The glucose nutrient solution was inoculated by soil microflora.

In the variants with clay, the resulting suspensions were agitated for 8 hours prior to the addition of mineral (biogenous) elements and inoculation with the microflora.

0.1, 1.0 and 3.0 percent of Na-bentonite, Na-kaolinite, Ca-bentonite and Ca-kaolinite suspensions were used. The fraction of particles less than 0.2 μ of the Brańany Bentonite and a commercial kaolinite were used.

The soil microflora inoculum was prepared as follows: 5 g of a chernozem soil were agitated in 500 ml distilled water at room temperature for 24 hours. The coarse particles were allowed to settle for two hours. 3.0 percent of the supernatant were used to inoculate the individual flasks.

Mineralization of glucose was studied in continually aerated flasks at 28 °C. The carbon dioxide evolved was determined daily by titration.

Growth curves of *Escherichia coli* were determined in batch culture without aeration at 37 °C. The experimental variant with 3.0 percent of kaolinite was omitted because of the rapid sedimentation of the clay

suspension and considerable analytical errors in the determination of counts. The growth curves were plotted from colony counts of *Escherichia coli* growing on the meat-peptone-agar.

RESULTS AND DISCUSSION

The results of glucose mineralization are summarised in Table 1. The daily mineralization rate of all the variants is shown. The effect of clay is different depending upon the type, the concentration and the kind of cations absorbed.

All the three concentrations of both Na- and Ca-kaolinite depressed the intensity of mineralization of glucose. This depressive effect rised with the increased amounts of the clay mineral. The greatest effect of kaolinite on the depression of mineralization was in the first and in the second day of incubation. This contributed substantially to the total decrement in mineralization. In following days, the substrate mineralization in kaolinite variants did not differ much from that of the control, at the end of the 12-day incubation being even higher than in the control variant.

The amount of glucose mineralized after the addition to the solution of 0.1, 1.0 and 3.0 percent Na-kaolinite was 81.60, 95.00 and 75.25 percent of glucose as compared to the control variant. The respective concentrations of Ca-kaolinite diminished the total glucose mineralization, as compared with the control, to 85.40, 86.40 and 73.20 percent. The concentration of 1.0 percent kaolinite was the least effective in the depression of substrate mineralization.

The effect of added bentonite on the mineralization of glucose is not as simple as that of kaolinite. Small concentrations of bentonite enhanced the mineralization of glucose. The 3.0 percent concentration of bentonite, on the contrary, depressed significantly the mineralization rate of glucose.

The most pronounced effect of added bentonite on the increasing rate of mineralization of the substrate was observed in the first day of incubation. At this stage also 3.0 percent bentonite increased the mineralization. 3.0 percent bentonite additions decreased carbon dioxide evolution, compared with the control in following days. On the contrary, the 0.1 and even more the 1.0 percent concentration of added bentonite slightly increased the amount of the mineralized substrate during the whole period of experiment.

The results indicated that the added clay exhibits the most pronounced effect in the first phases of incubation. A comparison of the effect of clays on the first steps of carbon dioxide evolution with the bacteria growth would be very interesting. Unfortunately, it is impossible to determine the growth curve of the overly complex soil microflora. For this reason the *Escherichia coli* culture was used in the following growth-curves studies. The nutrient media including clay additions were identical with these in previous experiments.

The data summarised in Figs 1—5 and in Table 2 indicate, that the added clays affected the growth curves chiefly in the lag-phase. The effect was similar to that in the glucose mineralization experiments. The two mono-

Table 1

The influence of various concentrations of bentonite and kaolinite on the mineralization of glucose by complex soil microflora (mgs of evolved carbon dioxide)

Days of incubation	Control (without clay)	Na-bentonite			Ca-bentonite		
		0.1 %	1.0 %	3.0 %	0.1 %	1.0 %	3.0 %
1	19.64	37.71	36.26	55.74	35.81	27.39	31.40
2	111.57	115.25	106.70	80.52	103.92	43.43	64.76
3	50.47	26.60	48.46	21.20	57.95	116.10	34.40
4	24.85	21.36	22.43	11.04	32.25	17.46	43.30
5	15.55	14.72	24.19	6.67	20.86	25.80	32.71
6	11.50	8.03	10.61	4.72	11.80	14.01	3.72
7	4.67	9.44	11.93	2.70	8.25	17.48	5.62
8	3.02	5.50	7.43	3.21	4.40	9.69	1.54
9	0.97	4.51	4.93	1.35	2.02	3.41	5.10
10	3.50	2.66	8.94	0.87	3.54	7.42	2.09
11	0.16	2.32	0.62	0.93	0.50	3.91	0.16
12	0.30	1.20	2.55	0.75	0.80	3.40	0.00
Σ mg CO_2	246.20	249.30	285.05	189.70	282.10	289.50	224.80

Days of incubation	Control (without clay)	Na-kaolinite			Ca-kaolinite		
		0.1 %	1.0 %	3.0 %	0.1 %	1.0 %	3.0 %
1	19.64	12.36	8.04	3.35	8.78	11.87	2.68
2	111.57	106.89	93.78	62.26	93.63	106.21	55.96
3	50.47	26.82	58.24	47.85	48.87	23.11	49.32
4	24.85	19.74	19.78	30.16	18.09	21.25	25.76
5	15.55	17.75	19.46	15.98	17.67	15.35	15.72
6	11.50	11.97	9.17	9.87	10.05	9.08	10.30
7	4.67	3.96	7.18	5.54	2.47	5.48	6.74
8	3.02	4.67	6.14	2.09	4.90	6.10	2.41
9	0.97	1.34	5.69	0.00	1.77	6.42	1.66
10	3.50	2.55	3.87	7.24	2.86	3.00	8.63
11	0.16	1.25	0.85	0.56	1.01	2.33	0.32
12	0.30	0.50	1.85	0.20	0.40	2.20	0.60
Σ mg CO_2	246.20	209.80	234.05	185.10	210.50	212.40	180.10

ionic forms of bentonic shortened the lag-phase of the growth curve of *Escherichia coli*. 1.0 percent concentration of both Na- and Ca-bentonite were most effective.

Lag-time of the growth curve of *Escherichia coli* in the control variant was 3.52 hours. The addition of 1.0 percent Na-bentonite lowered the value of lag-time to 1.2 hours and the same concentration of Ca-bentonite to 0.88 hour. The rate of growth and the growth yield were smaller than those in the control.

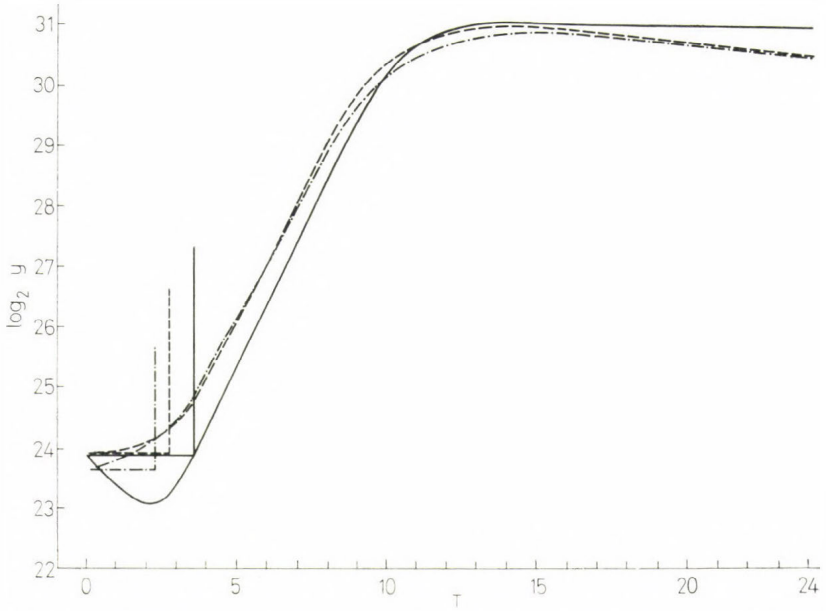

Fig. 1. The growth curve of *Escherichia coli*. ——— control without clay, — — with 0.1% Na-bentonite, — · — with 0.1% Ca-betonite, y = number of living cells, T = time (hours)

Table 2

Constants of growth curves of E. coli culture

Variants	Number of bacteria M · 10^6	Growth rate R	Lag time T_1	Growth lag L	Mean doubling time, g	Number of cell divisions per hour r
Control	2416	1	3.52	3.52	60′	1.00
0.1% Na-bentonite	2248	1.05	2.90	3.05	63′	0.95
0.1% Ca-bentonite	1901	0.90	2.23	2.01	63′	0.95
1.0% Na-bentonite	1562	0.70	1.20	0.84	84′	0.71
1.0% Ca-bentonite	1544	0.70	0.88	0.61	84′	0.71
3.0% Na-bentonite	2265	0.80	2.28	1.82	78′	0.76
3.0% Ca-bentonite	1518	0.75	1.45	1.01	84′	0.71
0.1% Na-kaolinite	1744	1.00	4.82	4.82	60′	1.00
0.1% Ca-kaolinite	2280	1.00	4.86	4.86	60′	1.00
1.0% Na-kaolinite	941	0.80	5.26	4.21	72′	0.83
1.0% Ca-kaolinite	984	0.80	4.78	3.83	75′	0.80

3.0 percent bentonite (Fig. 3) affected all the constants of the growth curve in a similar manner, but this influence was less pronounced.

The addition of kaolinite (Figs 4 and 5) depressed the growth of *Escherichia coli* just as unambiguously as the mineralization of glucose in previous experiments. In the presence of kaolinite, the lag-phase was prolonged,

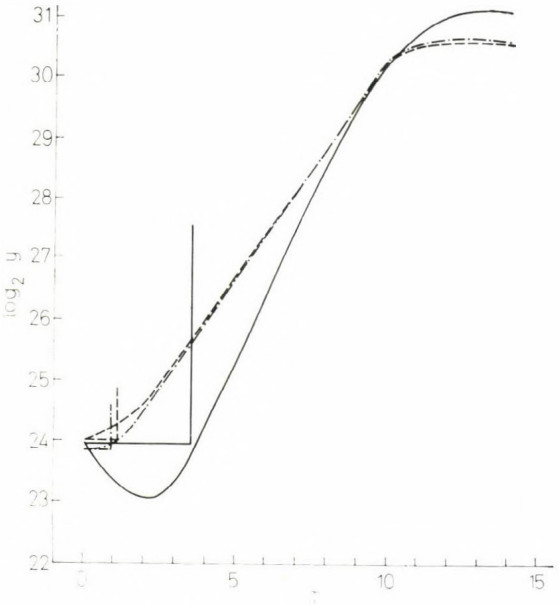

Fig. 2. The growth curve of *Escherichia coli*. ——— control without clay, — — — with 1.0% Na-bentonite, —·— with 1.0% Ca-bentonite, y and T = the same as in Fig. 1

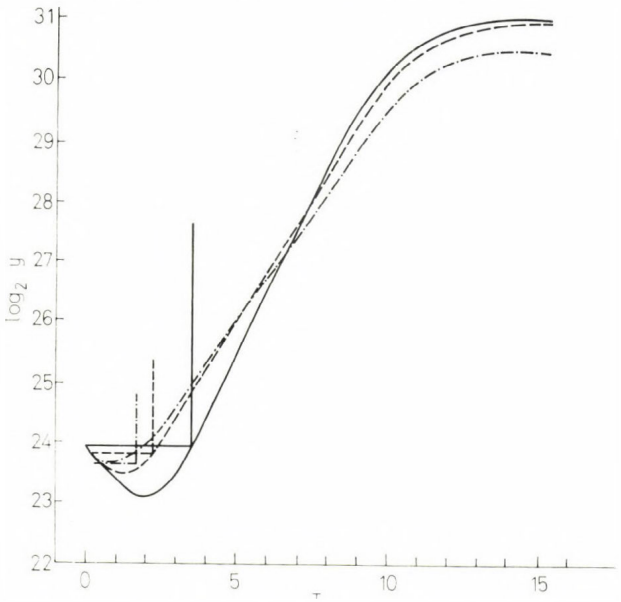

Fig. 3. The growth curve of *Escherichia coli*. ——— control without clay, — — — with 3.0% Na-bentonite, —·— with 3.0% Ca-bentonite, y and T = the same as in Fig. 1

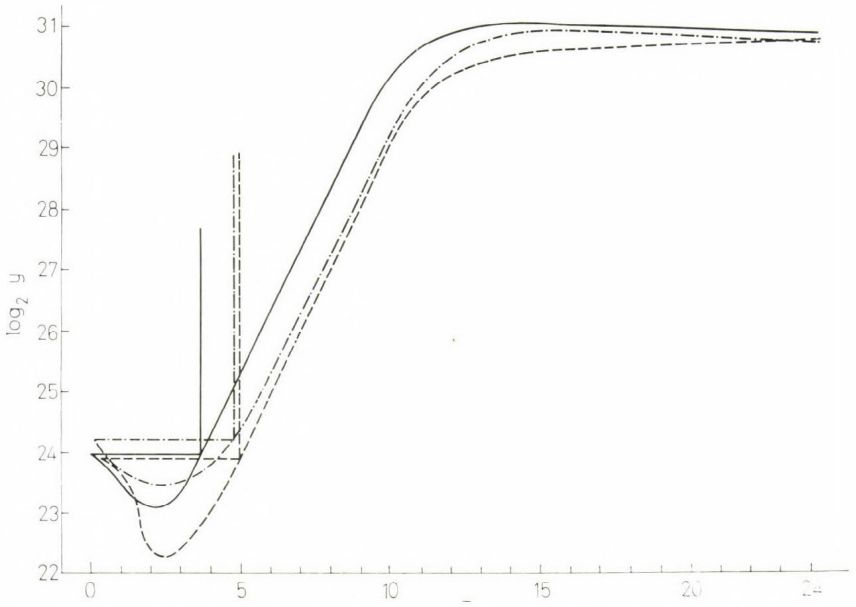

Fig. 4. The growth curve of *Escherichia coli*. ——— control without clay, — — — with 0.1% Na-kaolinite, — · — with 0.1% Ca-kaolinite, y and T = the same as in Fig. 1

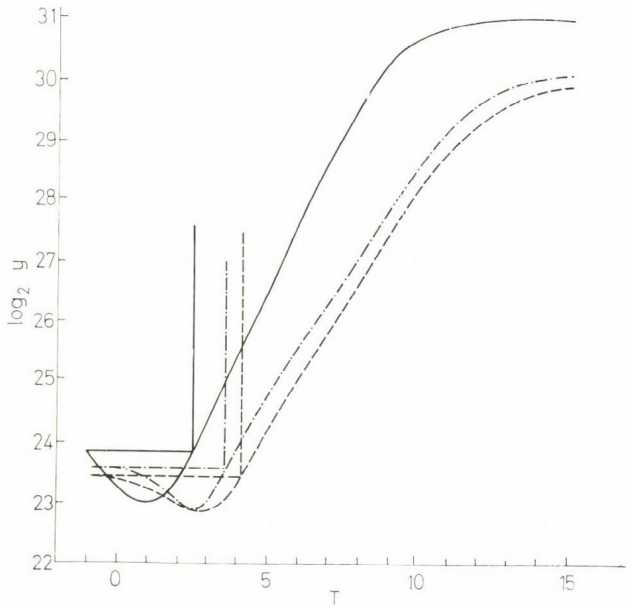

Fig. 5. The growth curve of *Escherichia coli*. ——— control without clay, — — — with 1.0% Na-kaolinite, — · — with 1.0% Ca-kaolinite, y and T = the same as in Fig. 1

the growth constants as lag time and growth lag were larger than in the check. Kaolinite depressed both rate of growth and the growth yield. The depressive effect increased with the increasing concentration of both forms of kaolinite. 1.0 percent Ca-kaolinite extended the lag-time by 0.86 hour, while 1.0 percent Na-kaolinite by as much as 2.14 hours as compared to the control.

In general, it was observed that the effect of clays was most pronounced at the start and during the initial period of incubation.

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HUMIFICATION OF A ^{14}C LABELLED ORGANIC MATTER IN SOIL AND THE INCORPORATION OF ^{15}N IN HUMIC SUBSTANCES

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There are obviously close relations between the autolysis of soil microbes and the synthesis of humic matters. But if in the course of cell autolysis humic matters synthetisized as expected, the process of humification has to be established in a far shorter time than usually accepted. On the basis of these considerations we attempted to accumulate microbial substances by adding glucose to the soil and to prove then, in the course of the induced autolysis of the cell bodies, the appearance of newly formed humic matters in the fractions concerned. Glucose was used because its carbon is assimilated totally in a relatively very short time by the soil microbes and with it the applied prime components soon disappeared in the soil medium. When radioactively marked glucose (1—6 ^{14}C) is used as carbon source, then ^{14}C must also be found in the fresh biomass. During the autolysis of these cell bodies when humic acid in fact has been developed, the ^{14}C can be detected in the form of new synthesized components in the humic acid fraction.

To obtain a fast accumulation of marked microbial bodies in soil, mineral substances were applied simultaneously, especially the nitrogen necessary for the formation of proteins. The nitrogen was marked too, so as to study its incorporation into the humic matter fractions for this purpose we used ^{15}N-ammonium sulphate. Preliminary tests have shown that ^{14}C and ^{15}N appeared in the humic matter fractions after a relatively short time. Therefore we began to analyse the initial humic matter synthesis by means of repeated analyses in short-time intervals (22 Erlenmeyer vessels (passing through of CO_2 free air with 25 °C in water bath) with each 4.2 ml of H_2O (approximately 60% water capacity) +360 mg of uniformly marked glucose ($s = 125$ μCig C_{Gluc}) + $(^{15}NH_4)_2SO_4$ ($c_a = 53.6\%$ ^{15}N) + 30 mg KH_2PO_4/60 g soil ($C_{Gluc} : N_{(NH_4)_2SO_4} : P : K = 144 : 14.4 : 6.8 : 8.6$); Analyses after 12, 15, 18 up to ... 75 hours; extractions and fractionations conventionally in $Fus_{(H_2SO_4)}$ = Fulvo-fraction out of the first acid treatment, Hus = humic acid fraction after extraction with NaOH and coagulation, $Fus_{(NaOH)}$ = Fulvo-fraction in solution after the precipitation of the humic acid, NeS = not extractable substances).

Under the given test and incubation conditions an intensive new formation of ^{14}C humic acid after 18 to 21 hours had already begun (Fig. 1). After approximately 42 hours a maximum of about 20 mg $^{14}C_{Hus}$ per 100 g soil was gained, followed by a decrease to approximately 14 to 15 mg after 75 hours. The contents of ^{15}N in the humic acid fraction showed a nearly analogous course. From this could be derived a common building in of these two nuclides into the new-formed humic matters. With that a narrow

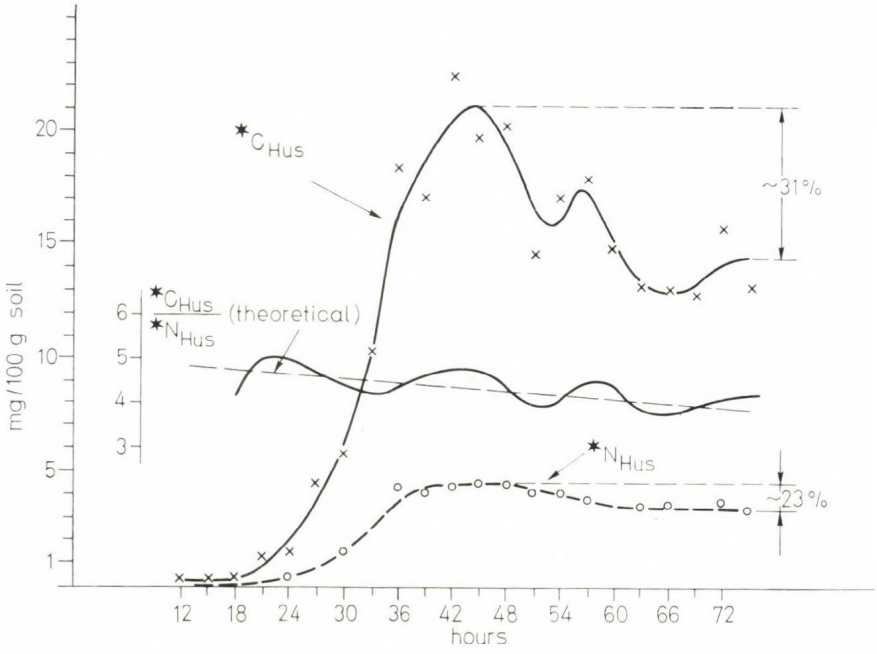
mg/100 g soil
20
15
10
5
1
*C Hus
~31%
*C Hus / *N Hus (theoretical)
6
5
4
3
*N Hus
~23%
12 18 24 30 36 42 48 54 60 66 72
hours

Fig. 1

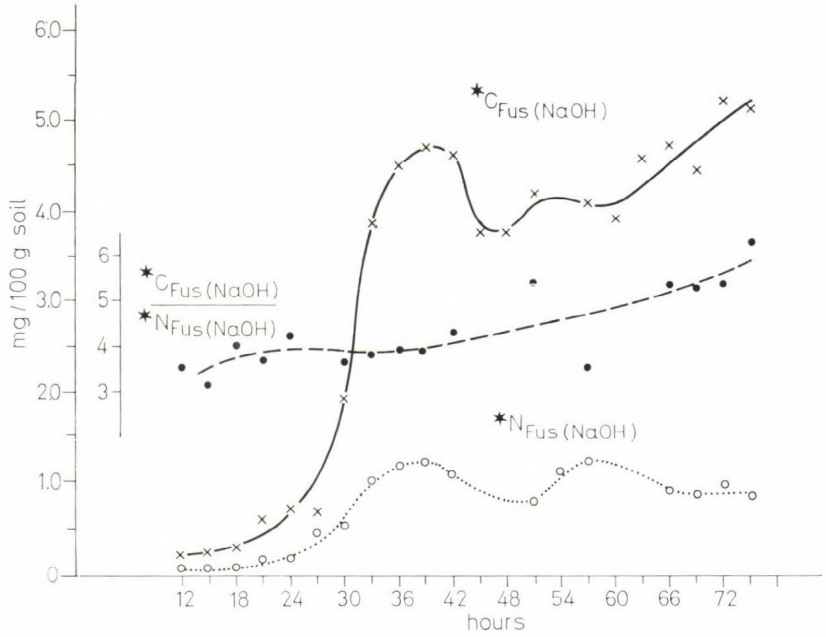
mg/100 g soil
6.0
5.0
4.0
3.0
2.0
1.0
0
*C Fus (NaOH)
*C Fus (NaOH) / *N Fus (NaOH)
6
5
4
3
*N Fus (NaOH)
12 18 24 30 36 42 48 54 60 66 72
hours

Fig. 2

$(^{14}C/^{15}N)_{Hus}$ ratio (nearly equal to 4.5) is reached, which is explained by a selective incorporation of N-rich compounds (aminoacids!) but also by a simultaneous taking in of $^{15}NH_4^+$ from the substrate into the humic acid.

The decrease of the amount of newly formed radioactive humic acids ($^{14}C_{Hus}$) after the maximum is very interesting. We assume that during this time, processes of mineralization in the medium began again and the biologically active assimilating new flora of microbes attack also the newly formed humic matters. By decreasing of $^{14}C_{Hus}$ the $(^{14}C/^{15}N)_{Hus}$ ratio in the fraction also changed; it narrowed coincidentally with the decrease of $^{14}C_{Hus}$, as shown in Fig. 1. This means a disappearance especially of N-poor humic acid components from the humic acid fraction. We suppose that during this time part of the high polymer fresh humic matters in the humic acid fraction became unstable by action of these secondary microbes and tended to a depolymerisation (especially in an alkaline medium as used during the analytical operations). From this it can be concluded that a certain part of N-poorer respectively C-richer components disappeared from the humic fraction and appeared in the course of the analysis in the lower polymer fulvo-fraction ($Fus_{(NaOH)}$). As seen in Fig. 2, the ^{14}C in this Fulvo-NaOH-fraction shows first also a maximum, analogous to the ^{14}C in the Hus-fraction. But during the dominant phase of these secondary mineralization processes in the medium we indeed obtained an increase of ^{14}C in the Fulvo-NaOH-fraction after 45 hours. At the same time an increase of the $^{14}C/^{15}N$ ratio in this fraction followed, effected by a change of the unstable, former ^{15}N-poor respectively ^{14}C-rich components of the Hus-fraction.

For calculation of the $^{14}C_{Hus}$ concentrations the total C amounts (C_{gesHus}) of the Hus-fraction had also to be determined. These total carbon amounts in the Hus-fraction, found with preliminary analysis, could have originated from the following components:

1) native old humic acids existing in the fraction already before the beginning of the investigations;

2) new-formed non-marked humic acids, developed out of native organic substances of the soil;

3) newly formed radioactive humic acids.

In spite of the far larger dispersion of the C_{gesHus} values, measured by elementary analysis, nevertheless a new incorporation of Hus in the fraction was to be expected in the form of an increase of the total carbon concentration, as seen in Fig. 3. By subtracting the radioactive ^{14}C parts from the C_{ges} amounts it was shown, that the newly formed Hus used the marked carbon source only; because (as seen in the figure) the inactive, native C-parts of the Hus-fraction remained constant during the whole incubation time. — This does not apply to the nitrogen in the newly synthesized humic acids. After formation of the difference ($N_{gesHus} - {}^{15}N_{Hus} = N_{Hus}$) the former level could no longer be reached during the phase of Hus-synthesis; this means that in the newly formed humic acids unmarked N originating from mineral or organic soil substances was also built in. Remarkable amounts of ^{14}C and ^{15}N applied in the form of glucose and ammonium sulphate also appear in the fraction of the nonextractable substances (NeS-fraction) as seen in Fig. 4. In this fraction ^{14}C and ^{15}N especially must have been obtained from newly synthesized microbial pro-

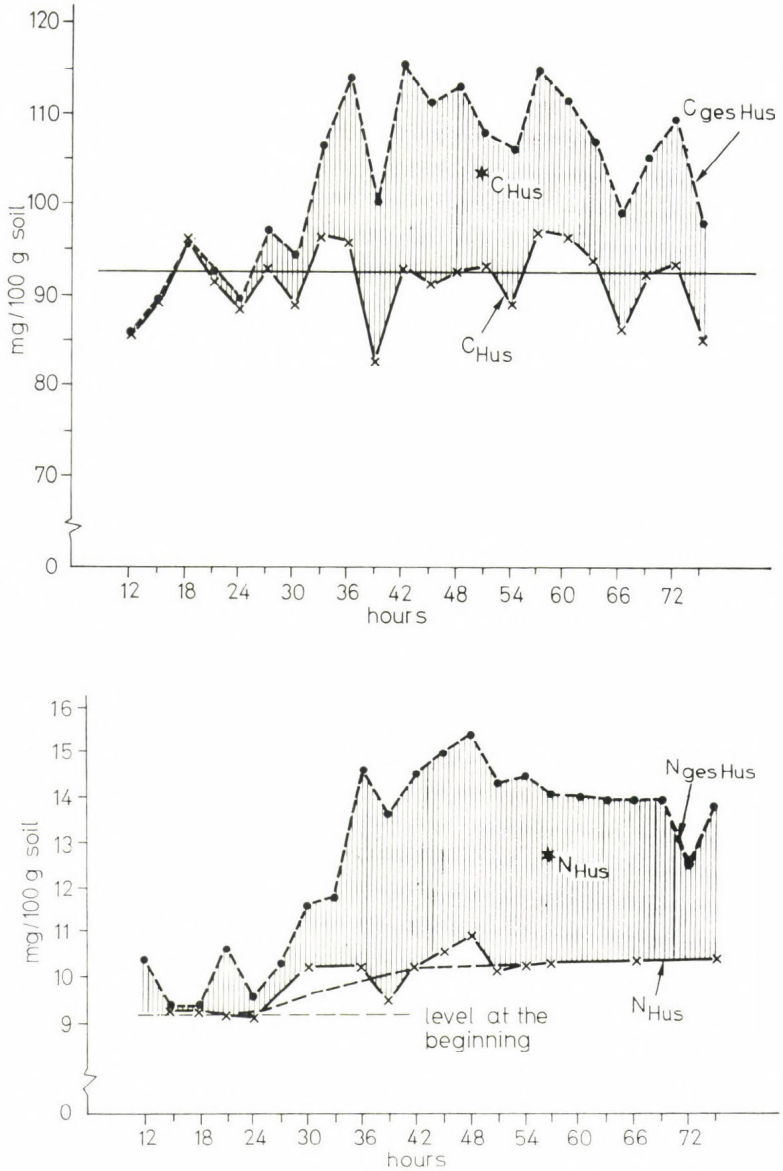

Fig. 3

tein. The C_{ges} and N_{ges} amounts in this fraction rising above the initial level were not formed from glucose—^{14}C and fertilizer—^{15}N only, but originated partly from both organic and in the case of N from mineral soil substances, as shown in Fig. 5.

These research results led us to make the following essential conclusions concerning the course of humification processes:

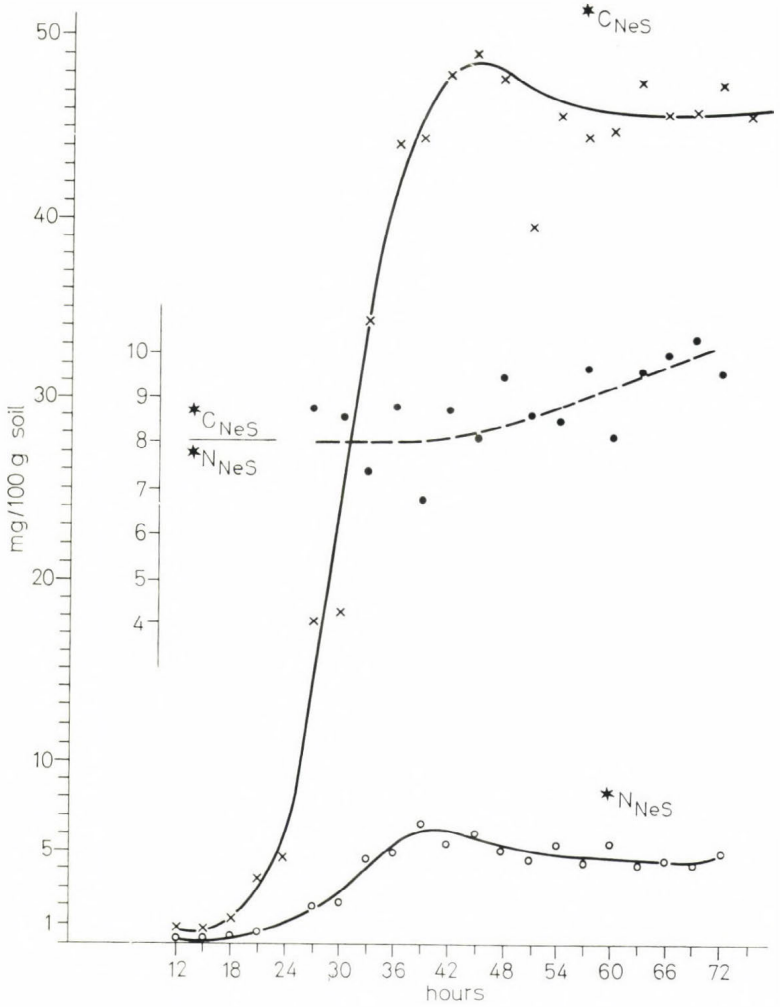

Fig. 4

1. The primary humification processes in soil run in very short periods and are in a close temporal coherence with the phases of the autolysis of soil microbes.
2. At the same time N is incorporated into the newly formed humic matters originating from both the soil humus and the applied mineral-type.
3. The newly synthesized, especially N-poor humic matters are not yet stable enough to resist the processes of mineralization of the substrate, and in course of this, they will be depolymerised (for instance back again to the fulvo-level).

An accumulation of humic acids in soil is obtainable as follows:

a) by stimulation of the humification potential (this means: rise of the synthesis maximum of humic matter by supporting the formation of bio-

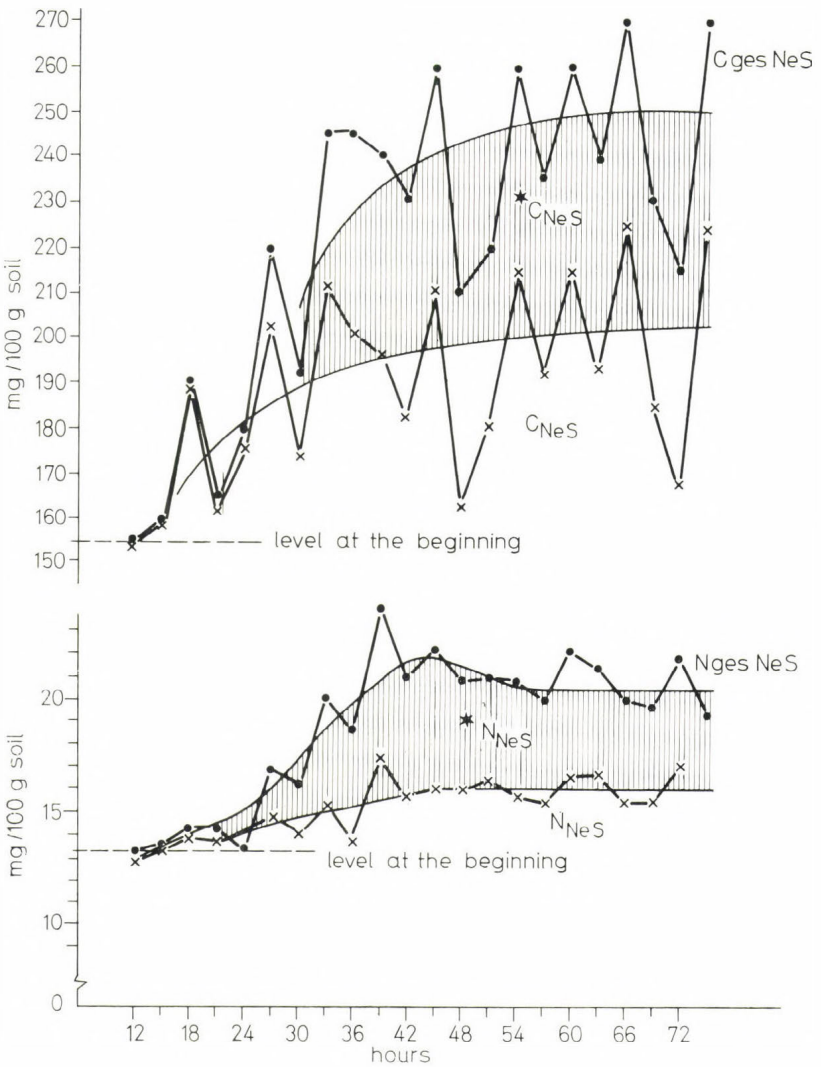

Fig. 5

mass and also by biochemical interferences in the course of autolysis).

b.) By inhibition of depolymerization of the newly formed humic acid (this means: the stabilization of newly formed products for example by building in of N, or also by protection in the course of ripening, i.e. by attaining a higher "irregularity" resp. a "randomised state" in the sense of the hypothesis by Dr. Kleinhempel).

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INFLUENCE OF MONTMORILLONITE ON METABOLIC PROCESSES OF THE HUMIC ACID FORMING FUNGUS *EPICOCCUM NIGRUM*

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Clay minerals form an important part of the solid phase of the soil. This colloid soil substance influences strongly the ecological system of microorganisms. The interactions between clay minerals and microbes are versatile in many respects. They influence, e.g. the sorption of organic and mineral nutrients, extracellular enzymes and other metabolic and autolytic products. Also the sorption of microbial cells on clay minerals may have some influence. According to different authors the addition of clay minerals to microbial culture media has some influence on microbial numbers and on microbial respiration. Also the utilization of nutrients may be sometimes enhanced.

If humus is considered as a product of microbial activity differences in the humification of organic materials might be expected as a result of the influence of clay minerals. Experiments of Filip (1968a, 1968b) for example showed an influence of bentonite on the formation of humic substances by a mixed flora of soil microorganisms. The addition of increasing amounts of bentonite to liquid, sand or soil cultures caused an increase of microbial numbers and in most cases also an increase in the formation of humic substances. Further experiments have been done to show the action of different clay minerals on some microscopic soil fungi under the aspect of humic acid formation. The present paper is restricted to a study of the influence of montmorillonite on the humic acid forming fungus *Epicoccum nigrum*. This fungus was shown by Martin et al. (1967), Haider and Martin (1967) to synthesize phenolic compounds from acetate and glucose units.

These phenolic compounds belonged mostly to resorcinol or hydroxycinnamic acid derivatives and were altered by the fungus introduction of further hydroxyl groups. They were autoxydized and polymerized to humic acids.

Epicoccum nigrum was cultivated in a liquid glucose-asparagine-culture medium which contained different amounts of montmorillonite ranging from 0.25 to 0.5 and 1 per cent. The flasks contained 300 ml of culture liquid and were inoculated with a suspension of spores of *E. nigrum*. They were incubated for 30 days at 22 °C either as steady or shaking cultures. In 10 days intervals parallels were analyzed for cell weight, glucose consumption and for different nitrogen fractions and for the formation of humic substances.

In Fig. 1 the cell weight after 10, 20 and 30 days of incubation is shown in the control and in the variants with different montmorillonite additions.

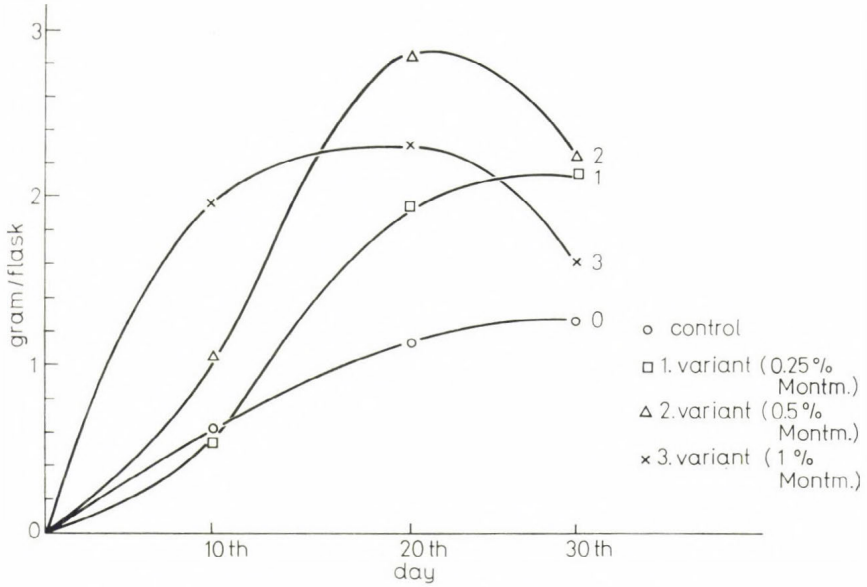

Fig. 1. Cell weight in steady cultures of *E. nigrum*

The values are average values from three flasks. One can observe that the highest montmorillonite concentration has the strongest effect on the initial cell growth. However, after 30 days of cultivation, the biomass is less than in the variants with lower montmorillonite content. The biomass in the control increases uniformly until 30 days, but never reaches the

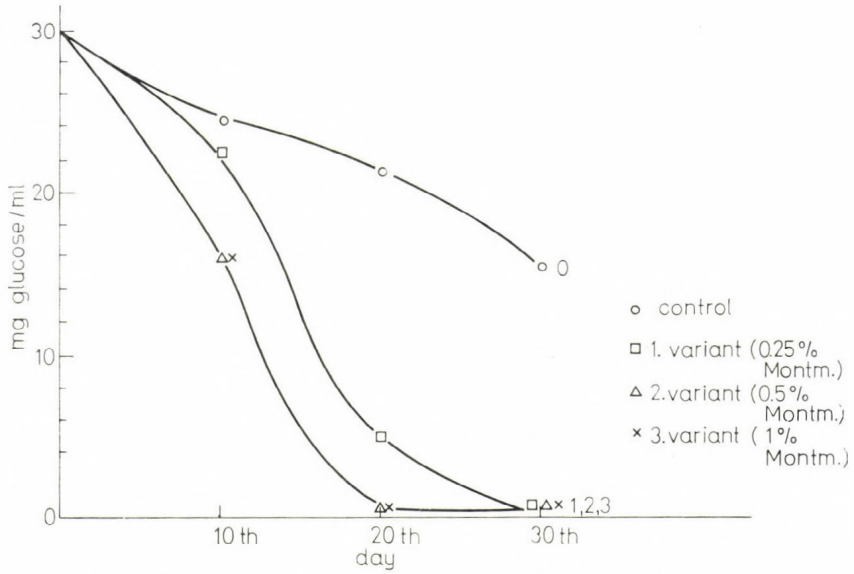

Fig. 2. Glucose consumption in steady cultures of *E. nigrum*

weight of the samples with montmorillonite. The decrease of the biomass after 20 days in the montmorillonite cultures indicated an earlier autolysis of the cells than in the control. This autolysis is caused by an acceleration of the nutrient consumption as shown in Fig. 2 for the glucose consumption. In the variants with 1% and 0.5% of montmorillonite the glucose is already consumed after 20 days, while glucose is still present in the control after 30 days.

The curves for the nitrogen consumption (Fig. 3) show a similar inclination; however the curves of the variants 1, 2 and 3 reach a minimum at between 20 and 30 days of incubation and then begin to rise. This rise is caused by ammonia and organic nitrogen compounds of low molecular weight, which are excreted during cell autolysis into the solution. The pH-values in the control decline from the initial value of 6 to about 4.5. During autolysis they rise to 7—8. The samples with montmorillonite show, according to the concentration, an accelerated decrease and increase in the pH-curve. With respect to cell growth, uptake of nutrients and release of nitrogen compounds during autolysis the shaking cultures behave in the same manner as the steady cultures. The nutrient solutions of *E. nigrum* in the controls change colour after about 15 days of incubation to red and after 20 to 30 days to a deep brown. The samples with montmorillonite addition change colour earlier and with increasing concentration of the mineral the colour becomes more concentrated. When the pH becomes alkaline, humic acids can be precipitated by addition of mineral acids. Fig. 4 shows the amount of humic acids in the control and montmorillonite variants. The height of the columns show the amount of the relevant humic acid per flask after 30 days of incubation. The values are indicated in the form of mg C as analyzed from aliquots of the humic acids. The first group of columns refers to the humic acid fraction which can be precipitated from the solution ($H.A._1$). The second group refers to the amount of humic acids which could be extracted from the cells directly with 0.5 n NaOH

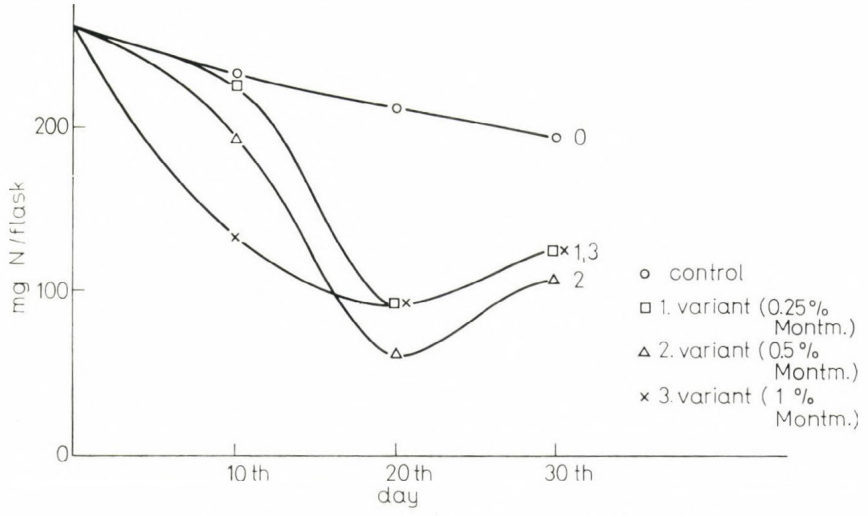

Fig. 3. Nitrogen consumption in steady cultures of *E. nigrum*

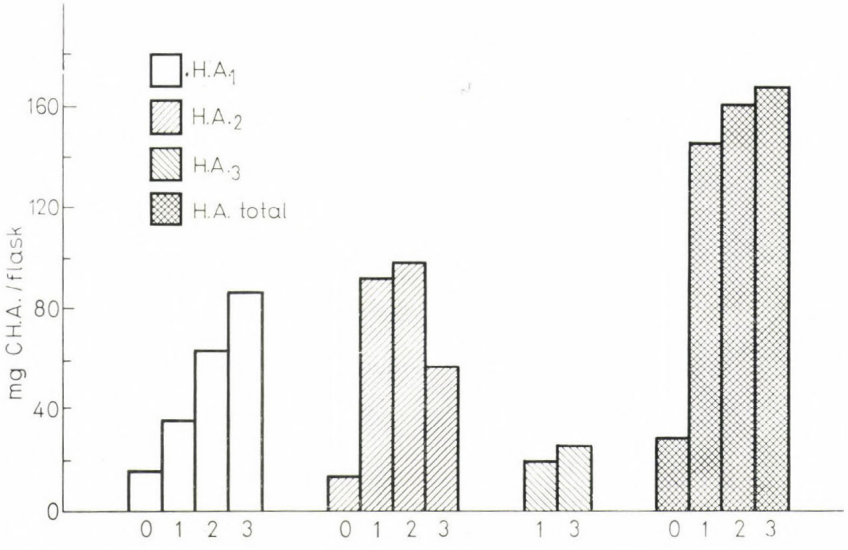

Fig. 4. Amount of humic acids in variants of experiments with *E. nigrum*

(H.A.$_2$). The third group gives the amount which can be extracted after previous hydrolysis of the cells with 1 n H_2SO_4 (H.A.$_3$). The last group of columns shows the total amount of humic acids (H.A.$_{total}$). The amount of precipitable humic acids increases with increasing montmorillonite concentration. Also a significant increase of the cell humic acids (H.A.$_2$) can be observed. However, no continuous increase in the quantity of humic acid in this fraction with increasing montmorillonite concentration can be observed, but a slight decrease in the highest concentration. With regard to the highest amount of humic acids in the solution of variant 3, it appears that the addition of montmorillonite causes only a higher formation of this acid fraction and accelerates eventually the excretion of humic acid precursors. Also the higher rate of autolysis and the accelerated consumption of nutrients may cause an increase of humic acid formation in the solution. Only small amounts of humic acids are released from the first and third variant by hydrolysis of the cells with sulphuric acid. The total amount of humic acids (in the last columns), increased significantly with increasing addition of montmorillonite. The nitrogen content of the humic acids after extraction of the ash amounts to about 7%. Also the C:N ratio of the humic acids is approximately 5 for all variants. This relatively high ratio is caused by a low C content of about 40% and a high N content of about 7%.

Furthermore the extinction measurement of the different humic acid fractions were referred to the same C content. Fig. 5 shows the negative logarithmic values of the extinction in the range of wave length from 475—700 μm for the humic acids isolated from the solution after 30 days of incubation. The specific extinction decreases in the following sequence: $0 > 1 > 2 > 3$. According to Kononova and other authors the extinction of humic acids is connected with its degree of condensation. The cell humic acids show the same tendency with regard to the specific extinction.

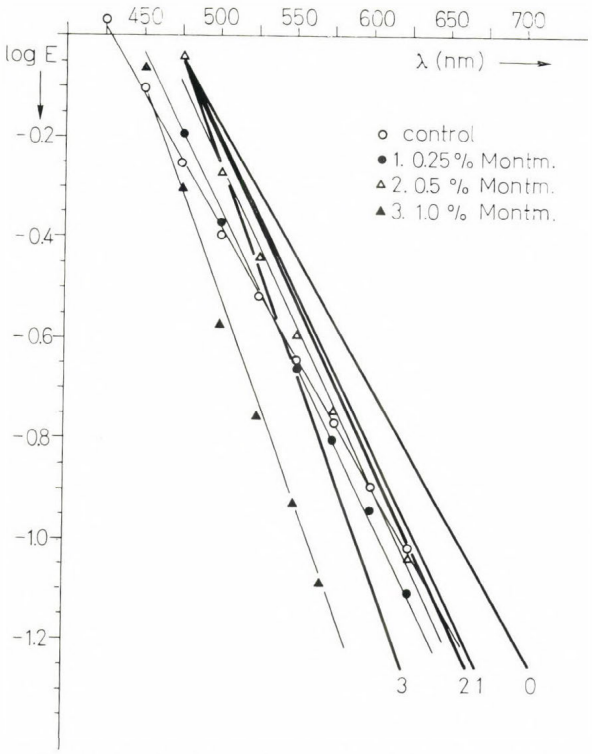

Fig. 5. Extinction values related to wavelength

As already indicated, the biomass of shaking cultures also increased in the same manner as in the steady cultures with increasing montmorillonite content. The absolute amount of biomass was even somewhat higher. Also the consumption of nutrients and the rate of autolysis were accelerated. However, from the nutrient solution of each variant no humic acids could be precipitated.

Increasing amounts of humic acids could be extracted from the cells using NaOH with increasing montmorillonite concentration. On the other hand, the amount of humic acids obtained after hydrolysis decreased. The total amount of humic acids was from five to seven times lower than in the corresponding steady cultures. It is still not possible to decide whether the better oxygen supply in the shaking cultures leads to a more complete respiration and to an increase in assimilation of the added nutrient into the biomass. Alternatively, the humic substances in the solution may not have a sufficient rate of condensation for precipitation. Available results give more credence to the first possibility. In summary, it can be said, that montmorillonite has a pronounced influence on the metabolism of *E. nigrum*. Similar effects were observed with other humic acids forming fungi.

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BIOGENESIS OF FRACTIONS OF FULVIC ACIDS IN CHERNOZEM SOIL

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The greatest problem in the investigation of humus substances is the elucidation of the pathways of its biogenesis whose specificity implies their particular role compared to other natural compounds. One of the main sources of the aromatic compounds is lignin whose "delayed" destruction under the action of microflora permits regarding it as a labile reserve of reactive "structural units".

General principles of the structure of fulvic acids, humic acids and lignin can be reliably established by the combined methods of pyrolysis and gas chromatography and comparison of the pyrograms obtained to detect identical components in the samples investigated. This method was used to investigate the samples of lignin, humic acids and fractions of fulvic acids obtained by column separation of fulvic acid filtrate (Dragunov et al. 1970). The scheme of fractionation of acid-soluble organic substances composing the fulvic-acid filtrate is shown in Fig. 1. The separation resulted in 13 fraction (a weighed sample of chernozem soil taken from the horizon A_0) which were identified according to data of element and functional analysis, gas chromatography of the products of pyrolysis, hydrolysis and oxidation as follows: fraction 1 — resin acids and their esters; fraction 2 — higher carbonic acids, paraffins and sterins; fraction 3 — was obtained in trace amounts and therefore was not studied; fraction 4 — polysaccharides consisting of glucose and pentose links as well as of glucuronic acids; fraction 5 — phenol glucosides; fraction 6 — compound of a peculiar character (proper fulvic acids); fraction 7 — resin acids; fraction 8 — resin acids and condensed aromatic compounds; fraction 9 — polysaccharides of the polyuronic-acid type; fraction 10 — the system of phenol glycosides of a marked acid nature, fractions 11, 12, 13 — compounds of a peculiar chemical nature (proper fulvic acids).

Thus, the employed technique allows separation of proper fulvic acids from concomitant compounds of inspecific character.

Pyrolysis and gas-chromatography was carried out as reported elsewhere (Mursakov et al. 1970) (Fig. 2).

Comparison of the pyrograms obtained indicates the presence of common main components in pyrolysates of lignin, humic acids and the fractions 6 and 12 of fulvic acids in chernozem soil. This is also evidenced from Table 1. Pyrogram of fraction 6 is similar to that of humic acids except that there are more peaks in the chromatogram of humic acids, which indirectly indicates the complex structure of humic acids. Pyrograms of the fractions 12 and of humic acids also evince a number of common components, yet fraction

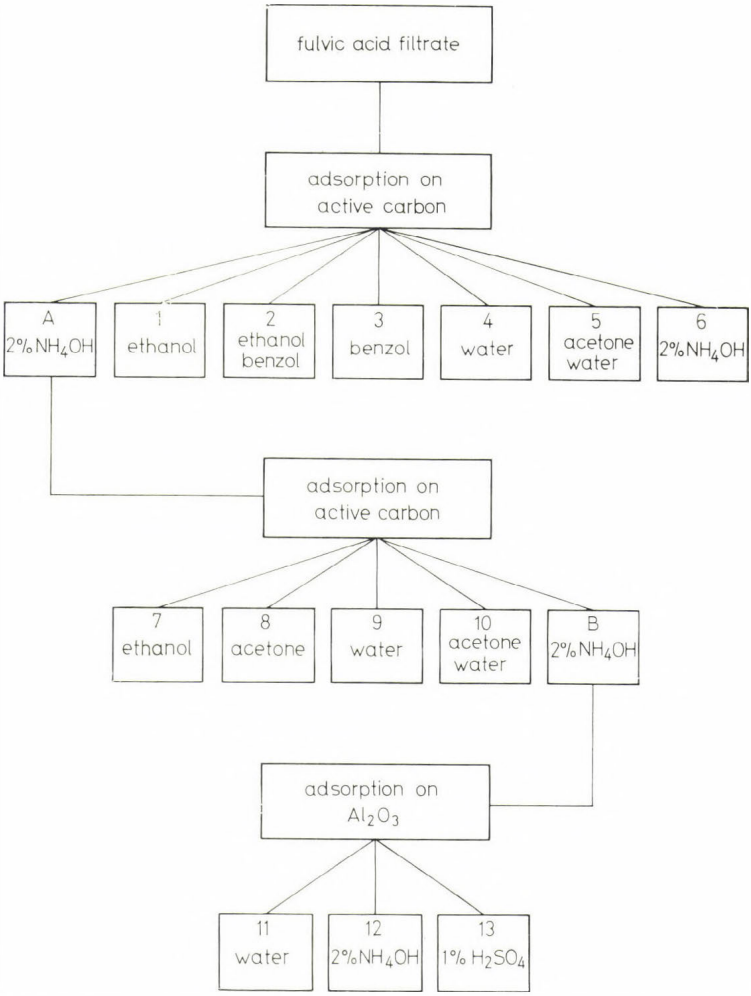


Fig. 1. A scheme of fractionation of the fulvic acid filtrate of chernozem

12 contains more volatile compounds than fraction 6. These findings allow us to suggest tentative pathways of lignin transformation to humus substances.

Organic substances entering the soil apparently undergo multiphase destruction. At the first stage, oligomers of lignin are formed, whose further transformation proceeds by two ways: 1) decomposition with formation of inspecific substances and "structural units" of fulvic acids; 2) interaction with reactive compounds of another origin with formation of polycomponent system of humic acids. "Structural units", that are formed at the first stage, interact with carbohydrate and protein fragments and form fulvic acids tentatively included in fraction 6. The part of humic acids which is formed at the first stage from oligomers of lignin is stabilized

through isomerization and goes over to fulvic acids composing fraction 12 as well as to unspecific substances. This scheme is in good accord with the heterogeneity of fulvic acids and humic acids as well as with the presence of a lot of substances of unspecific nature.

Therefore, there is a close genetic relationship between organic compounds of plants and qualitative new substances — humic substances of the soil. The crucial role in the process of transformation of plant materials belongs to microorganisms.

Soil microflora plays an important role not only in the synthetic processes but also in decomposition of newly formed substances. Under certain conditions, utilization of humic substances is carried out by the complex of microorganisms among which the major role is played by bacteria. Among the published earlier reports, of most interest are the investigations on narrow fractions of fulvic acids and humic acids because of the possibility thereby of studying specificity of separate groups of microorganisms against the compounds of various chemical nature.

To investigate decomposition of separate fractions of fulvic acids under the action of mixed microflora of the soil, we resorted to incubating the media containing fulvic acid preparations with inoculated soil microbes. At present, the major criterion for the extent of decomposition of humic substances is the per cent of decolourization according to which determination is made of the residual amount of the substance. However, from our viewpoint, the use of this method cannot be regarded as precise and the numerical values thus obtained, accurate. As evidenced from the literature, the molecules of humic substances contain numerous functional groups, including chromophores:

$$>C=C<, \qquad >C=N-, \qquad -N=N-, \qquad >C=O;$$

of special importance being the formation of quinoid structures. The action of chromophores is enhanced by auxochromes whose role is played by polar groups such as $-OH$ and $-NH_2$. The colouration of chemical compounds can be substantially affected by the factors observed during incubation of microorganisms in the media with humus substances. Hence, microbial destruction involves concealment of changes in the colouration and photocolorimetrical measurements of colour indices do not reflect all processes occurring in the culture medium. Apparently, a new criterion more precisely reflecting the essence of the process of decomposition of humus substances is necessary. As one of such criteria, can be regarded the determination of per cent changes of carbon in the control and treatments. Diminution of the carbon content can be associated with release of volatile compounds: CO_2, lower carbonic acids, alcohols, etc. We believe that the amount of volatile components can be regarded as a reliable sign of the accessibility of a given substance for microorganisms. Actually, decomposition of the organic substance resistent to microbial action involves breaking of but a small amount of accessible bonds while aromatic nuclei are decomposed slowly. Therefore, the amount of volatile components would be insignificant which might be directly related to the weak effect of decomposition.

Recently, destruction of the complex organic substances by microorganisms has become an accessory method for investigating their chemical nature. As evidenced from analysis of our data, the preparations have dif-

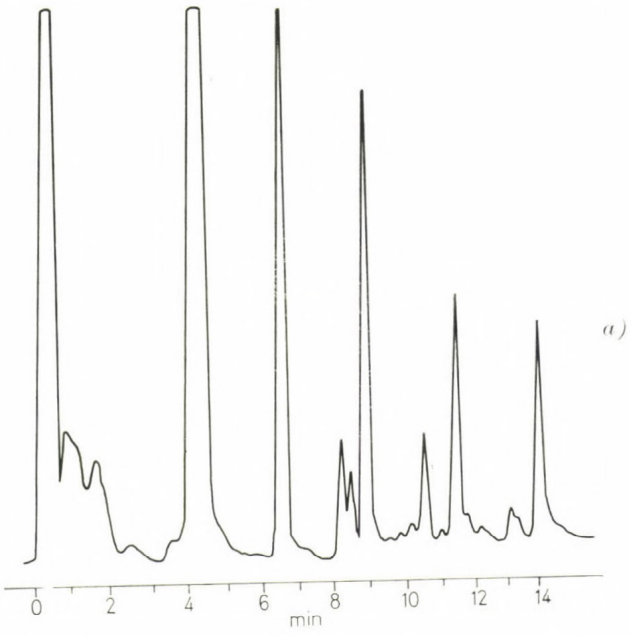
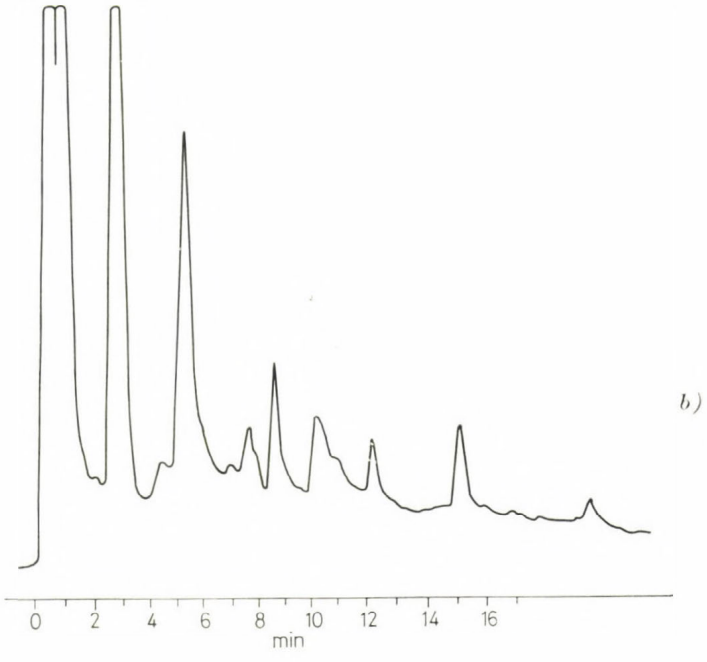
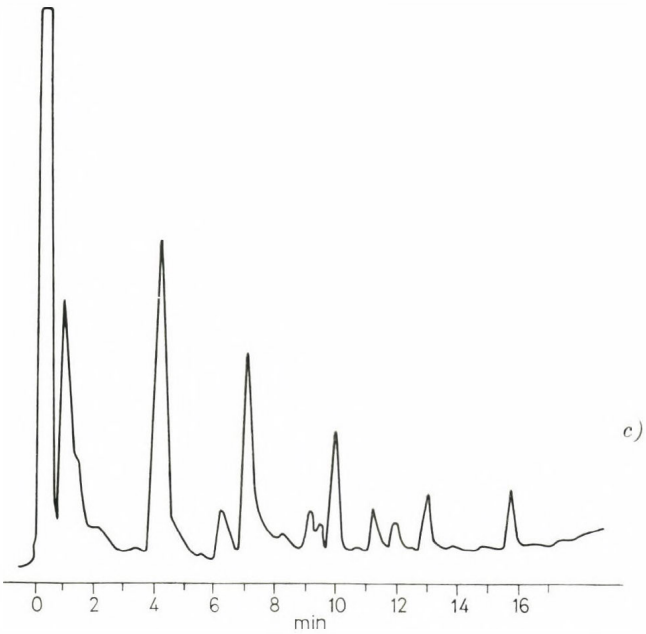


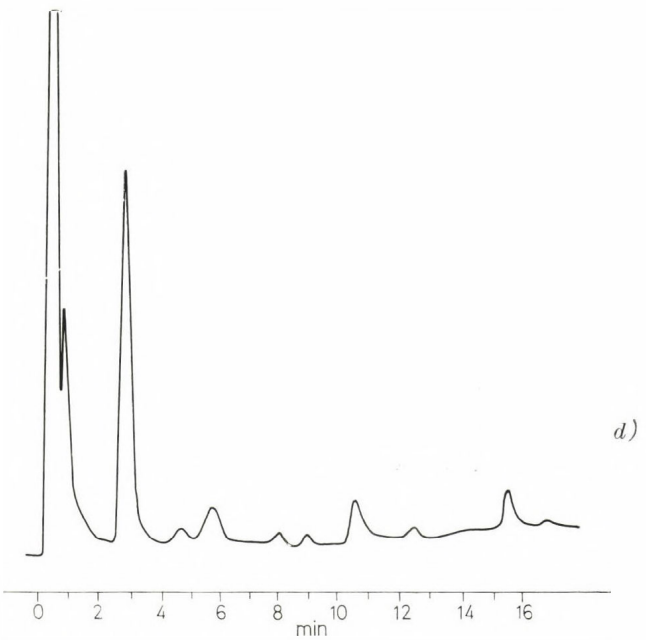
Fig. 2. Chromatograms of the products of pyrolysis of: *a)* lignin; *b)* fraction 6





c)

c) fraction 12; *d)* humic acids of chernozem



d)

ferent stability against separate groups of microorganisms; in the presence of some compounds, the pattern of developing microflora is rather uniform and includes trivial groups of microorganisms while other preparations result in the appearance of heterogeneous character of microflora including rare forms. This observation is in good accordance with data on the chemical nature of the fractions studied. However, the method based on determination of the carbon content does not take into account the changes that are connected with formation of substances not removed from the range of reaction. To get the qualitative assay of these changes, other procedures are required.

As a tentative procedure, we propose to carry out comparison of the products of pyrolysis of the medium before and after growing of microorganisms. The products of pyrolysis were fixed with the use of gas chromatography. This procedure was employed due to the fact that development of microorganisms involves changes in the chemical nature of the substances constituting the culture medium; pyrolytic decomposition, which is a complex physico-chemical process, creates specific character for the test and control samples which are manifest in chromatograms.

To illustrate the applicability of the method of pyrolysis-gas chromatography in microbiological studies, we employed fraction 7 (Fig. 3) which is least decomposed by microbes (as judged from the per cent of the carbon content changes). Pyrograms of the control and test samples demonstrated marked differences thus indicating the possibility of establishing deep qualitative differences in the composition of organic substances.

Thus the presented data prove that microorganisms actively participate in the decomposition of humus substances, while various groups of soil organic substances posses different stability against microflora. It can be proposed that differences in the microbial patterns of soil are directly connected with quantitative distribution of various fractions of fulvic acids as the most active portion of the humus.

An important evidence of biogenesis of humus substances can be obtained from investigation of their optical activity. It is known that optical activity is one of the most universal characters of natural compounds. Optically active substances of the soil were investigated earlier for obtaining data concerning the use of spectropolarimetry in cosmic biology (Blei and Liskowitz 1965). Acids and alkali extracted organic substances about 10 times as much, yet the number of optically active compounds is not proportional to the number of organic compounds. Water extracts possess optical rotatory dispersion like alkaline ones and produce levorotation. The curve of dispersion of optical activity of the acid extract is similar to that of proteins in acid solution while alkaline extracts give a dispersion curve analogical to nucleotides. The character of the curve of rotational dispersion indicates that it might belong rather to monomeric nucleotides: polymers show complex curves with alternating minimums and maximums and has nothing in common with the curves of the soil extracts (Fresco et al. 1961). The curves of optical rotatory dispersion of the soil extract are the sum of optical activities of individual compounds. Therefore it is of interest to investigate the optical activities of narrower fractions.

We carried out spectropolarimetric investigation of fractions of fulvic acids of chernozem isolated by the above method. Determination of optical

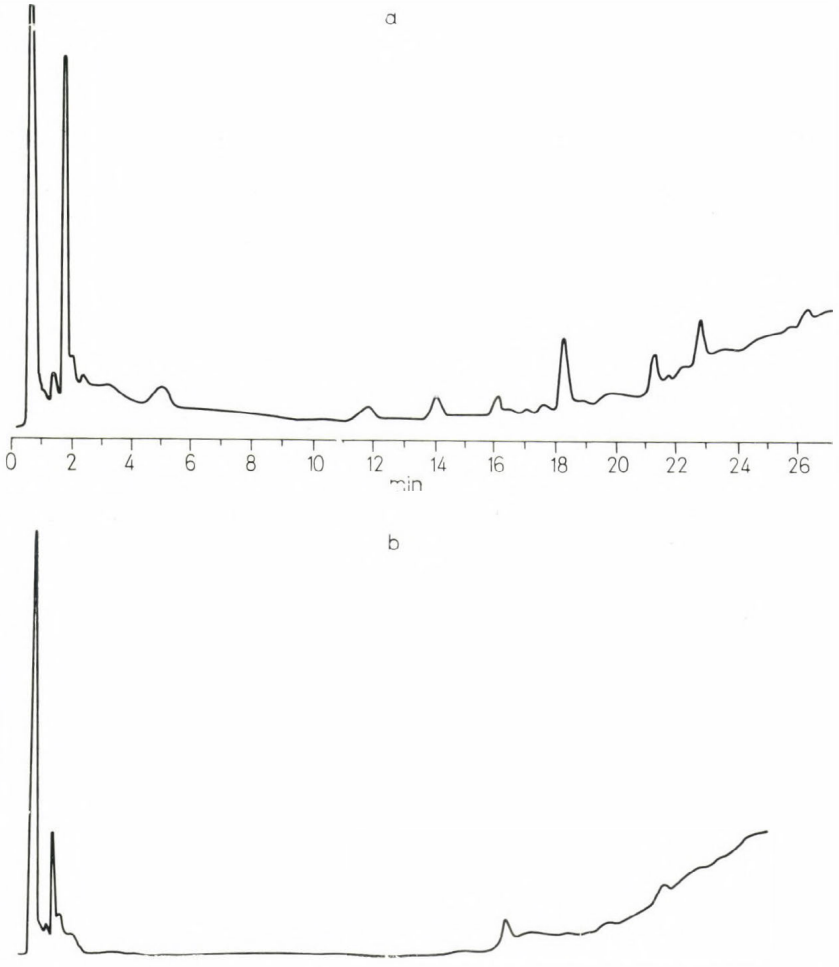


Fig. 3. Chromatograms of the products of pyrolysis of the medium containing fraction 7 of fulvic acids of chernozem: *a)* before the experiment; *b)* after the experiment

activity of eluates was carried out in UV-range of the spectrum. This investigation permitted establishing that fractions 1, 5, 6 and 9 possess dextrorotation while fractions 2, 4 and 12, levorotation.

Interpretation of the obtained data with regard to the biochemistry of humus formation might be as follows: either optically active substances compose the fractions, or they are involved in stereospecific reactions with formation of optically active compounds, or they undergo decomposition by the soil microflora resulting in accumulation of one of the isomers in greater amount, or these compounds are the product of decomposition of microbial masses. Of definite interest is the occurrence of optical activity in fractions 5 and 12, which can be regarded as representatives of proper fulvic acids, yet their dispersion curves are of different character. Thus,

the occurrence of optically active organic compounds in the soil is the most important argument for the decisive role of biogenous factors in the process of formation of humic substances.

To summarize, a suggestion can be made that humification is the process associated with deep transformation of all compounds that enter the soil and hence result in the formation of qualitatively new compounds with concomitant simple substances — products of destruction of the initial and newly formed materials. The soil microorganisms play the major role in all stages of formation of soil organic compounds including the compounds of specific nature.

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THE EFFECT OF SOIL BIOCHEMICAL FACTORS ON THE TRANSFORMATION OF HUMIC SUBSTANCES

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The suitability of our method for following the transformation of humic substances is established by the investigation of various soil types from 7 European countries. All the results received by the author with this method showed on the one hand the differences of soil genetics and on the other the possibility of applying the mentioned investigations in the evaluation of the effect of soil biochemical factors on humus quality. The results of these investigations are summarized in several works by the author (Hargitai 1955, 1964, 1965, 1967). The main essence of this method is the optical investigation of the NaF and NaOH extracts for determination of the ratio of their extinctions which characterizes the stability and at the same time the quality of humic substances. The more humified and with humic substances of a good quality more closely bonded Ca^{2+} ion has generally higher stability numbers which are calculated as follows:

$$Q = \frac{E_{NaF}}{E_{NaOH}} \qquad \text{from this the}$$

$$K = \frac{E_{NaF}}{E_{NaOH} - H} \qquad \text{stability coefficient can be calculated}$$

E_{NaF} means the extinction of 1% NaF extract
E_{NaOH} means the extinction of 0.5% NaOH extract

These coefficients characterize not only the soil types with greater differences, but at the same time the transformation of humic substances too.

This transformation can be effected through the change of soil biochemical processes, which is initiated by different cultivation, fertilization and manuring methods. We have investigated the effect of the different cultivation methods (deep, normal, shallow) and also the effect of fertilizers and manures on the transformation of the humus quality through the biochemical processes of the changed soil.

Results summarized in Table 1 show very well, that the greatest stability coefficients can be determined by normal fertilization in all the variations of cultivation. As expected, normal manuring decreases the stability coefficients by increasing the content of raw organic substances. The determined grade and value of humification is much lower in such cases.

A strong fertilization increases the stability coefficients, but less than a normal fertilization. Remarkable decrease of the stability coefficients is only produced by normal and strong manuring. The results compared

Table 1

Effect of cultivation and fertilization on the transformation of humic substances expressed in stability coefficients

Cultivation	Control	Normal			Strong		
		Fertilized	Manured	Fertilized and manured	Fertilized	Manured	Fertilized and manured
Shallow	0.754	0.852	0.702	0.725	0.809	0.708	0.658
Deep	0.744	0.896	0.639	0.570	0.582	0.509	0.601

with deep cultivation treatments show very similar results but all the numbers are slightly lower.

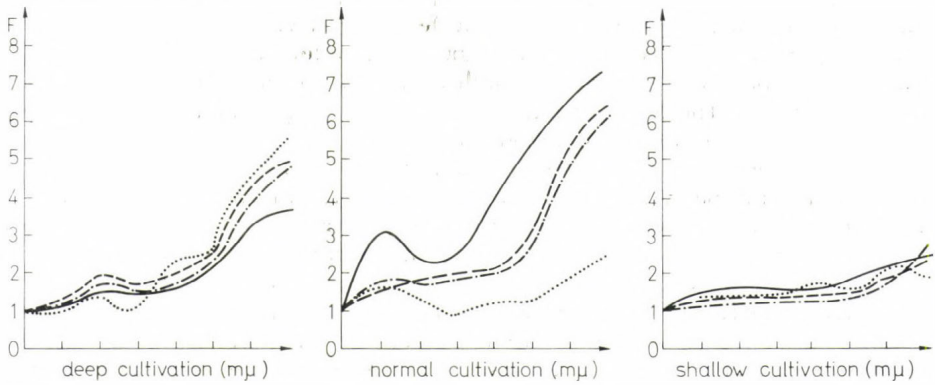
The transformation of humic substances is influenced by the cultivation methods, which change the equilibrium of aerobic and anaerobic processes. According to several investigations and our results (Flaig 1954, Hargitai 1955, 1963) a few years ago, the exact evaluation of extinction curves was found to be the most suitable for characterization of the humus quality transformations.

This is possible with the determination of F curves. F curves are first mentioned by Hock (1936) and some investigations of the author were based on these a few years ago. They can be calculated from the extinction values divided by the extinction of the humus extract measured by the longest length with the Pulfrich photometer. When this calculation is carried out in each case, with all the extinction values, the received F values can be expressed depending on the wavelength in the F curves.

The various types of F curves characterize the kinds of humic substances. This fact was expressed in our work a few years ago (Hargitai 1963). Analysis of the F curves showed the great effect of cultivations in developing characteristic feature from the curves independent of the quality of extracts.

It is very interesting, that a greater condensation of humic substances and in connection with this a high increase in the colour curves (in the

Fig. 1. F curves of humic substances (NaF extracts)



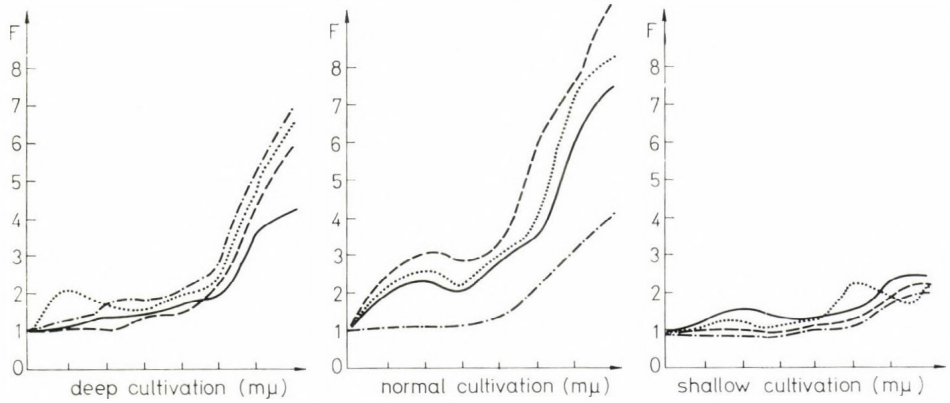


Fig. 2. F curves of humic substances (NaOH extracts)

F curves) is found in the results very markedly, when the field is normally cultivated. The soil studied was a brown forest soil. These curves clearly depend on aerob-anaerob conditions. The transformation of humic substances under different cultivations continues as a result of various microbiological and soil biochemical processes. These processes are indicated in the colour change of humic substances expressed in the F curves (Figs 1 and 2).

Summarizing, it can be stated, that our method for determining the stability coefficients is first of all, suitable for characterization of the effect of fertilization of humic substances.

The change in the equilibrium of aerob-anaerobic conditions effected by cultivation is expressed by the transformation of humic substances measured with the F curves according to our method.

SUMMARY

There is a complicated interaction between biochemical processes in the soil and changes in the humus quality. The latter has an influence on physico-chemical properties, on nitrogen and nutrient element dynamics of the soil. Its determination is very important. Our investigations a few years ago illustrated a more favourable action of the combined application of aeration and liming together, than the total of their separated action.

Our method developed from the method of Hock to determine the humus quality through stability coefficients not only estimates genetic differences but also the changes in the humus quality required. From the many interesting investigations we have selected in this paper only a few; namely the effects of surface and deep cultivation and the results of various organic and mineral fertilizer applications.

The main essence of these results is as follows:

1. The grade of humification illustrated with the stability coefficients is greater by surface cultivation than by deep cultivation.

2. Greater amounts of nutrient elements assured by fertilizers have an excellent effect on humification. A greater supply of microelements makes the transformation in the humus quality possible probably due to greater activity of microorganisms.

The change of humus quality can be determined by the F curves calculated from the extinctions in NaF and also in NaOH extracts through distribution with the extinction of the longest wavelength.

The F curves are suitable for more detailed characterization of humification processes and for estimating the changes in humus quality affected by soil biochemical factors.

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DECOMPOSITION OF METAL-HUMIC ACID COMPLEXES BY MICROORGANISMS

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Recent research concerning the decomposition of organic substances in biolithic caustics draws attention to the technological development in the preparation of organic mineral fertilizers using microorganisms.

We have tested organic mineral mixtures with microorganisms known earlier as active in relation to sodium humate decomposition. For example, *Pseudomonas sinuosa*, *Mycobacterium citreum* oligonitrophil №. 13 B, *Actinomyces* № 4, *Mucor plumbens* were studied in the Department of General and Soil microbiology of the Ukrainian Academy of Sciences, Institute of Microbiology.

The experiment and data based on the quantity of movable forms of nitrogen are shown in Table 1. From experimental results, the increase of movable forms of nitrogen were observed (especially during the two weeks of composting); water soluble carbon in all the experimental variants was found to decrease. Further composting showed a decrease of movable nitrogen but indicated no absolute return to the original first sample level.

In fertilizer mixtures, the organic matter of peat was decomposed, the result of which led to the increase of both nitrogen and carbon movability. In relation to this decomposition the microbiological cultures of *Actinomyces* № 4, *Mucor plumbens* and mixed cultures *(Pseudomonas sinuosa*, oligonitrophil, *Actinomyces*, *Mucor plumbers*, *Mycobacterium citreum)* were active.

It was established that upon addition of transition metals into the mixture of salt, the fertilizing qualities of the mixtures were increased. Therefore a detailed investigation of humic acid decomposition in complex with metals was carried out.

Complex-forming ability of peat, brown coal, and humic acid was proved by physico-chemical and purely physical methods.

Interest in these complex compounds is due to their stability, the latter can be explained by formation of cyclic structures in which metal is firmly bonded. They can possess properties which are peculiar to metal or ligand.

Humic acid was extracted from the peat of Ukrainian origin (village Zamglai) by means of sodium pyrophosphate with sodium hydroxide and then purified by a benzenealcohol mixture. Then it was thoroughly dialysed. The solutions were saturated with copper, iron, and calcium from chlorinated salts. Next followed the consequent treatment of the samples with water, potassium chloride, and 0.1 N nitric acid in order to get samples with different types of bonds of metal with humic acid. The permanent treatment by these reagents made possible the removal of

Table 1

Content of nitrogen forms in peat mixtures with various

Variant N	Experiment	Content in % of easily hydrolyzed nitrogen without nitrate recovery				
		2 days	2 weeks	1 month	2 months	3 months
W 1.	Peat from Zamglai	0.24	0.29	0.27	0.29	0.27
2.	Peat + Ammonia water + cement dust + Phosphate slag + Corn extract + *Pseudomonas sinuosa*	0.51	0.61	0.53	0.56	0.57
3.	ditto + *Mycobacterium citreum*	0.51	0.60	0.58	0.58	0.57
4.	ditto + oligonitrophil № 13 B	0.52	0.62	0.57	0.59	0.61
5.	ditto + *Actinomyces № 4*	0.45	0.68	0.59	0.65	0.66
6.	ditto + *Mucor plumbens*	0.53	0.64	0.63	0.66	0.65
7.	ditto + mixture of cultures	0.52	0.61	0.63	0.61	0.56

excessive metallic ions bonded with humic acid by absorption forces and ion bonds.

Research performed by the ESR method (electron spin resonance) showed that the metal was coordinated by humic acid 9.

The signal intensity of free radical type in humic acid is decreased whereas metal concentration increases. This is the result of the double bond saturation with metallic ions in the process of complex formation. Complex formation takes place in direct contact of humic acid with metal and does not depend upon the prevailing processes of ion substitution and hydroxide formation. The forming of higher complexes (when the signal of the free radicals of humic acid disappears altogether) is stable and even the treatment of 0.1 N HNO_3 does not lead to the destruction of the complex and to the liberation of the metal.

Treated by a preparation of humic acid, sand was enriched with cultures of microorganisms. The mixture was subjected to a constant temperature treatment and then analysed for water soluble carbon and unbonded metal. The obtained data are shown in Tables 2, 3, 4 and 5.

The joining of metals to humic acid did not lower the rate of acid decomposition. During the process of fermentation the growing quantity of humic acid and carbon was transferred into the water extraction whereas the copper content decreased. The mentioned decrease was in good ratio with the increasing number of microorganisms to permit the inference that the microorganisms themselves absorbed the missing copper. In the second sampling, the iron content in the water extraction at first increased, but then decreased. Humic acids may have penetrated into the cells of microorganisms together with the metal.

In such a way, the presence of complex forming — humic acid — removed for microorganisms metallic toxity in corresponding doses.

microorganisms according to terms of fermentation (laboratory tests)

Content in % of easily hydrolyzed nitrogen with recovery of nitrates					Content of overall nitrogen in %			
2 days	2 weeks	1 month	2 months	3 months	2 days	1 month	2 months	3 months
0.25	0.29	0.30	0.29	0.27	3.16	3.17	3.19	3.09
0.55	0.65	0.61	0.60	0.59	3.61	3.57	3.63	3.51
0.59	0.66	0.65	0.63	0.62	3.63	3.56	3.52	3.56
0.58	0.64	0.65	0.63	0.61	3.64	3.48	3.44	3.48
0.58	0.70	0.68	0.67	0.68	3.53	3.47	3.50	3.44
0.57	0.70	0.70	0.68	0.66	3.67	3.46	3.51	3.42
0.52	0.70	0.64	0.66	0.58	3.65	3.71	3.60	3.52

Table 2

Content of humic acid in mg/kg in the water extraction when decomposing Ca-humic acid

Microorganisms	Ca-humic acid	Per 2nd day	Per 17th day	Per 37th day
Pseudomonas sinuosa	Control	214	574	549
	H_2O	217	331	657
	KCl	301	392	498
	HNO_3	209	481	—
Mycobacterium citreum	Control	150	657	401
	H_2O	249	759	667
	KCl	227	544	640
	HNO_3	224	273	274
Actinomyces № 4	Control	250	546	231
	H_2O	301	799	463
	KCl	320	495	144
	HNO_3	220	355	410
Mucor plumbens	Control	280	677	280
	H_2O	237	625	29
	KCl	248	358	170
	HNO_3	284	377	225

Table 3

Content of humic acid in mg/kg in the water extraction when decomposing Cu-humic acid

Microorganisms	Cu-humic acid	Per 2nd day	Per 17th day	Per 37th day
Pseudomonas sinuosa	Control	214	574	549
	H_2O	332	510	288
	KCl	286	421	499
	HNO_3	217	712	401
Mycobacterium citreum	Control	150	657	401
	H_2O	248	426	370
	KCl	301	393	116
	HNO_3	300	679	359
Actinomyces № 4	Control	250	546	341
	H_2O	265	326	556
	KCl	282	567	311
	HNO_3	207	446	298
Mucor plumbens	Control	280	677	483
	H_2O	187	608	802
	KCl	236	421	522
	HNO_3	296	427	528

Table 4

Content of humic acid in mg/kg in the water extraction when decomposing Fe-humic acid

Microorganisms	Fe-humic acid	Per 2nd day	Per 17th day	Per 37th day
Pseudomonas sinuosa	Control	214	574	549
	H_2O	173	687	610
	KCl	243	391	243
	HNO_3	274	723	607
Mycobacterium citreum	Control	150	657	401
	H_2O	210	786	373
	KCl	270	514	932
	HNO_3	230	540	790
Actinomyces № 4	Control	250	546	341
	H_2O	377	538	522
	KCl	284	572	2
	HNO_3	250	325	560
Mucor plumbens	Control	280	677	483
	H_2O	93	272	261
	KCl	63	280	536
	HNO_3	94	578	576

Table 5

Content of copper in mg/kg in the water extract when decomposing Cu-humic acid

Microorganisms	Cu-humic acid	Per 2nd day	Per 17th day	Per 37th day
Pseudomonas sinuosa	Control	—	—	—
	H_2O	15	11.6	4.1
	KCl	37.5	34.24	7.0
	HNO_3	39.0	37.12	5.3
Mycobacterium citreum	Control	2	—	—
	H_2O	31.0	28.4	5.9
	KCl	44.5	33.1	7.2
	HNO_3	35.0	9.8	9.0
Actinomyces № *4*	Control	—	—	—
	H_2O	34.5	28.1	9.9
	KCl	43.5	30.0	11.2
	HNO_3	42.2	10.0	10.0
Mucor plumbens	Control	—	—	—
	H_2O	37.0	27.6	10.0
	KCl	34.5	31.6	11.8
	HNO_3	39.0	11.7	7.7

SUMMARY

On the effect of microorganisms *Pseudomonas sinuosa, Mycobacterium citreum, Actinomyces* №. *4, Mucor plumbens*, the decomposition of humic acid complexed with Ca, Fe, and Cu took place.

In the process of constant temperature treatment of complexes with microorganisms, metals were released to finally bond them to the microorganisms.

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METABOLISM OF AROMATIC COMPOUNDS IN SOIL

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Aromatic structures represent a part of humic and lignin compounds. It is therefore assumed that processes of their transformation could occur also during the decay of native soil organic matter and plant residues in soil. With regard to the importance of microbial transformation of aromatic monomers within the frame of total changes of soil organic matter, we attempted to characterize in detail the course of these processes under the complex conditions prevailing in soils. Special attention was paid to the possible influence of some environmental factors on the microorganisms involved.

The rate of oxidation of particular aromatic compounds in the soil may differ. Quinic acid was oxidized most rapidly, the lag phase of the oxidation of other substances was prolongated in the sequence *p*-hydroxybenzoic acid, vanillin and coumarin.

Only one peak was observed in the rate of oxygen uptake for the oxidation of some compounds, e.g. quinic acid, *p*-hydroxybenzoic acid, and salicylic acid. Two or more peaks, within the concentration range from 2 till 20 micromoles of substrate per 1 g of air-dry soil, appeared in the case of benzoic acid, coumarin and vanillin. The appearance of more peaks on the oxygen consumption rate curves may indicate the sequential induction of enzymes participating in the oxidation of particular intermediates or the succession of different types or groups of microorganisms.

More attention was paid to the study of vanillin decomposition because of current incidence of this metoxylated phenolic aldehyde in the structure and metabolic changes of lignin and humic substances. The biochemical reactions such as oxidation, demethylation and aromatic ring fission are also included both in vanillin and lignin degradation pathways.

On the basis of the oxygen consumption rate curve it may be assumed that in the oxidation of vanillin in soil suspension during the first peak aromatic intermediates were oxidized. The second peak corresponded to the oxidation of non-aromatic compounds. This presumption was supported by amount of oxygen consumed and by the results of chromatographical analysis of the ether extracts of soil in the respective phase of the process. The occurrence of vanillin, vanillic acid, and protocatechuic acid successively was determined. No aromatic conpounds were found after the last peak was started.

The data obtained by the Stanier's technique of simultaneous adaptation were found to be in agreement with the results of chromatographic analysis. It may be therefore concluded that vanillin is decomposed in the soil via

vanillic acid and protocatechuic acid before the aromatic ring is opened. The possible connection between the metabolism of vanillin and p-hydrobenzoic acid in soil might be supposed.

Both total number of bacteria and relative incidence of bacteria utilizing vanillin as the only source of carbon and energy increased during the vanillin decomposition in soil. Twenty-one strains of bacterial vanillin decomposers were isolated. From this set 15 strains were identified as *Pseudomonas sp.*, 5 strains as *Cellulomonas sp.*, and 1 strain as *Achromobacter sp.*

Different strains of bacteria, isolated from soil, might oxidize vanillin in different ways as shown by the shape of respiration curves.

1) The curve of the rate of oxygen consumption for the oxidation of vanillin by *Pseudomonas sp.* (V 2) revealed two peaks. The first peak corresponded to the oxidation vanillin to vanillic acid according to the findings of the chromatographic analysis and amount of oxygen consumed. The second peak was due to the oxidation of vanillic acid and following aromatic and non-aromatic intermediates. Total amount of oxygen consumed was equal to 70% of the theoretical amount required for the complete oxidation of the given amount of substrate to carbon dioxide and water.

2) Under the same conditions the strain *Cellulomonas sp.* (V 9) oxidized vanillin to vanillic acid only. It was confirmed also by the findings of chromatographical analysis and by the oxygen uptake measured.

3) The respiration curves obtained in pure culture of *Cellulomonas sp.* (V 12) was sigmoid, with one peak only. However, the total amount of oxygen consumed was equal to 60% of the theoretical amount required for the complete oxidation of the given amount of substrate to carbon dioxide and water.

Environmental conditions might distinctly influence the oxidation of vanillin. The experiments with washed cell suspensions of *Cellulomonas sp.* revealed that the optimum pH for the oxidation of vanillin to vanillic acid was 6.5. The lag phase of the respiration curve in the further oxidation of vanillic acid was shortest at pH 7.5. Vanillic acid was not oxidized at pH 8.5 during the 13 hours of incubation, vanillin was not oxidized at pH 9.1. The influence of clay minerals on the decomposition of aromatic monomers in soil was also observed. Both dioxide production and oxygen consumption during the oxidation of vanillin were accelerated in relation to the increasing amount of montmorillonite added.

The addition and following decomposition of source of carbon and energy in the soil may influence the amount, composition and metabolic activity of the microflora taking part in another metabolic process and consequently the course of the transformation of that compound. These interactions are usually very complicated and they may be brought about at different levels.

The enrichment of soil with glucose led to the acceleration of the vanillin decomposition in soil. The similar effect on vanillin oxidation in soil was observed also with ribose, fructose, glutamic acid, pyruvate and malic acid. The pretreatment of the soil with glucose shortened the lag phase of the oxidation not only in the case of vanillin but also of quinic acid, *p*-hydroxybenzoic acid and coumarin. The oxygen consumption for the oxidation of fulvic acids representing the fraction of native soil organic

matter with significant aromatic character was distinctly accelerated after 20 hours preincubation of the soil with glucose.

The technique of simultaneous adaptation and chromatographic analysis indicated that the biological decomposition of fulvic acids involved aromatic monomers such as vanillin, vanillic acid, *p*-hydrobenzoic acid, and protocatechuic acid.

It was shown that the amendment of soil with glucose, ribose, fructose, glycine, glutamic acid, pyruvate and malic acid led not only to the general proliferation of microflora in soil but also to the increase in relative incidence of specific vanillin decomposers.

In order to find the possible explanation of preferential proliferation of this physiological group of microbes the effect of glucose on the vanillin decomposition in washed cell suspensions of bacterial vanillin decomposers was investigated. In contrast to the well known influence of glucose described as catabolite repression or inhibition it was observed that the presence of glucose might induce the formation of certain enzymatic systems involved in the aromatic substrate decomposition.

While the strain of *Cellulomonas sp.* oxidized vanillin during 6 hours only to vanillic acid, in presence of glucose also the second peak of oxygen uptake arose, i.e. vanillic acid was further oxidized. Glucose influenced neither the oxidation of vanillin to vanillic acid nor the oxidation of protocatechuic acid which is considered to be the next intermediate following vanillic acid in this metabolic pathway. It could be assumed that the glucose stimulated probably the oxidative demethylation of vanillic acid.

On the basis of data presented the following general conclusions may be drawn: The metabolism of aromatic compounds in the soil is related to the density and potentials of microorganisms involved. The activity and succession of particular groups of microorganisms are affected by the complex of environmental factors. The enrichment of soil with glucose or other sources of carbon and energy might influence the decomposition of aromatic substances not only through the increase in total microbial counts but also by affecting their metabolism, by the induction of specific enzymatic systems which could result in preferential incidence of aromatic substrate decomposers. The analogy between the effect of glucose on the ability of the soil microflora to utilize aromatic substances and the role of the microflora in the "priming effect", in which enrichment of the soil with glucose leads to the intensification of the mineralization of native soil organic matter, could be assumed.

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MICROBIOLOGICAL INVESTIGATIONS ON SPRUCE RAW HUMUS

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In the mountain regions of the GDR the spruce forests, as a rule, grow on soil with low lime content, the nutrient supply of which is poor to medium. Under these conditions the dead organic substance is not decomposed quickly enough, only to an inadequate extent and consequently the nutrients fixed in the raw humus do not become readily available to the higher plants, and the stand growth is often inhibited. The acceleration of the metabolism is in the main a problem of soil microbiology. The soil microorganisms can be activated by adequate fertilization measures. Though the microbiology of forest soils was investigated already by Fehér (1933), forest fertilization experiments were hitherto evaluated microbiologically only to a limited extent. Fiedler and Fiedler (1961) as well as Fiedler *et al.* (1963) checked the effect of stand liming on the microflora of spruce raw humus. Papers by Loub (1959), Schneider (1959) as well as Franz and Loub (1959) dealing with soil-biological investigations on fertilization experiments with lime and nitrogen are available. A supplementary report is given on two trials with spruce raw humus, the objective of which consisted in investigating the effect of the nutrients Ca, N and P as well as of a differentiated liming with and without NPK—fertilization. One experimental plot (trial I) lies in the Bärenburg district (eastern part of the Erzgebirge) within the site of the moderately fresh Altenberg quartz-porphyry podzol. It belongs to the fairly poor site unit group with average water supply at medium altitudes and moist climate. The soil is poor in plant nutrients, especially phosphorus. In the year of the investigation the spruce stand was about 70 years old. The variants and fertilizers applied are presented in Table 1. Calcium was given in the form of lime with 63% CaO, N in the form of calcium ammonium nitrate with 25% N and P as Thomas phosphate with 16% P_2O_5 content respectively.

The second experimental plot (trial II) is situated in the Ilmenau/Gehren forest enterprise on the crest Langer Berg (800 metres above sea level) in the Thuringian Forest. The more than 100-year-old spruce stand grows on an iron-humus podzol which developed from Frauenbach quartzite in Pleistocene layers. The liming experiment carried out in combination with NPK fertilization on a thick raw humus layer, comprises the variants presented in Table 3. As for the applied slag sand and metallurgical lime, it deals with Ca silicates of equal chemical composition, but different granulation.

Particulars of the trial layout as well as of the trial itself were described by Mai and Fiedler (1968, 1969a, b, c) Of the numerous trial results only extracts can be presented here.

Table 1

Amount of nutrients incorporated into the ploughed layer of soil in trial I

Variants of experiments (control)	Active substance of fertilizers, kg/ha
Control	—
Ca	3140 kg CaO
N	165 kg N
CaN	3140 kg CaO + 165 kg N
NP	165 kg N + 175 kg P_2O_5
CaNP	3140 kg CaO + 165 kg N + 175 kg P_2O

RESULTS

1. Chemical investigations. Tables 2 and 3 show some chemical characteristics of both experimental plots.

Table 2

Main chemical characteristics of the soil in trial I

Variants of experiments (control)	pH (KCl)	Total C content, %	Total N content, %	C/N ratio	Nitrate-N (in mg N/100 g of dry soil)
Control	2.9	47.9	1.71	28.0	0.7
N	3.2	47.6	1.71	27.8	1.0
Ca	4.7	44.1	1.70	25.9	2.5
NP	4.6	44.0	1.65	26.7	5.9
CaN	4.8	43.0	1.69	25.4	7.2
CaNP	5.6	40.1	1.62	24.8	7.7

Table 3

Main chemical characteristics of the soil in trial II

Variants of experiments (control)	Ca* dt/ha	pH (KCl)	Total C content, %	Total N content, %	C/N ratio	Ammonium-N (in mg N/100 g of dry soil)
Control	—	2.7	50.8	1.42	35.8	6.8
Slag sand	57	3.5	31.6	0.86	37.0	6.8
Coarse	114	3.8	29.3	0.86	34.4	2.4
Metallurgical	57	4.6	43.1	1.28	33.7	3.8
Lime, ground	114	5.2	37.0	1.08	34.4	4.2
Hydrated	57	5.6	38.1	1.18	32.3	2.5
Lime	114	5.5	33.5	1.08	31.0	4.0

In trial I the pH values increased markedly by lime as well as by phosphorus fertilization. The single treatment with calcium ammonium nitrate increased the pH values only slightly. For the variants Ca, NP, CaN and

CaNP the carbon content was significantly lower than for O and N. The slight decrease of the carbon content in some variants was not significant. The C/N ratio diminished in accordance with the decrease of the C content. Nitrate-N was found in the untreated plots only in very small quantities. Fertilization with calcium ammonium nitrate did not also increase significantly the nitrate-N content; its effect, however, in combination with a lime and phosphate was good.

In trial II lime fertilization increased the pH-values depending on form and quantity of lime. The C and N contents as well as the C/N-ratios decreased. The greatest effect was always obtained with hydrated lime, the weakest effect with coarse slag sand. The ammonium-N content significantly decreased in all variants except that of the lowest slag sand dosage. On this experimental plot nitrate-N was found in considerably less quantities than in trial I. The quantities were so small that they could no longer be exactly ascertained. A slight increase of the NO_3—N content was recorded especially after treatment with hydrated lime.

2. Numbers of bacteria, fungi and *Actinomycetes:* The actual and relative numbers of the groups of microorganisms are presented in the Tables 4 and 5.

In trial I the bacterial numbers in all variants increased markedly after fertilization. Compared with the control the increase was in all variants — except single N fertilization — statistically significant. The best effect was produced by the fertilizer combinations NP and CaNP. *Actinomycetes* were rarely found on untreated plots. In all variants, especially CaNP, their number increased. In this trial the actual numbers of the microscopic soil fungi did not change significantly. However, the relative portion of fungi in the total number of germs diminished in all fertilizer variants.

Table 4

Numbers of microorganisms in trial I in the F_1-horizon (indicated in 10,000/g of dry soil)

Variants of experiments	Bacteria		Fungi		Actinomycetes	
	$\bar{X}$	%	$\bar{X}$	%	$\bar{X}$	%
Control	119	79.3	30	20.0	1	0.7
Ca	639	94.9	26	3.9	8	1.2
N	284	86.3	43	13.1	2	0.6
CaN	800	95.6	28	3.3	9	1.1
NP	1563	97.1	41	2.5	6	0.4
CaNP	1467	96.9	29	1.9	18	1.2

In trial II the thick raw humus layer of the untreated plots contains considerably less bacteria than that of trial I. Liming led to an increase in the number of bacteria, especially when metallurgical and hydrated lime were given. The numbers of microscopic soil fungi decreased in most variants significantly. The relative ratio of fungi in the total number of germs decreased to a very great extent. Liming led to a considerable increase in the number of *Actinomycetes.*

Table 5

Numbers of microorganisms in trial II in the F_1- and H-horizons (indicated in 10,000/g of organic substance)

Variants of experiments	Ca applied dt/ha	Bacteria		Fungi		Actinomycetes	
		$\bar{X}$	%	$\bar{X}$	%	$\bar{X}$	%
Control	—	29	38.7	44	58.7	2	2.7
Slag sand,	57	87	58.8	33	22.3	28	18.9
coarse	114	96	56.8	28	16.6	45	26.6
Metallurgical	57	177	75.0	24	10.2	35	14.8
lime ground	114	228	85.4	15	5.6	24	9.0
Hydrated	57	210	78.1	25	9.3	34	12.6
lime	114	204	80.9	19	7.5	29	11.5

Table 6

CO_2-release in laboratory of material taken from the F-horizon in trial I and II (indicated in mg CO_2/g of dry soil in 24 hours)

	Variants of experiments in trial I					
	Control	Ca	N	CaN	PN	CaNP
CO_2 mg/g/day	2.5	3.7	2.7	2.7	3.9	4.1

	Variants of experiments in trial II						
Ca applied, dt/ha	Slag sand, coarse			Metallurgical		Hydrated	Lime
				lime ground			
	Control	57	114	57	114	57	114
CO_2 mg/g/day	1.3	1.5	1.7	2.5	3.4	3.2	3.7

Table 7

Decomposition of cellulose at the boundary between the A_1-, A_2-horizons (in % of the parent substance)

	Variants of experiment in trial I					
	Control	Ca	N	CaN	PN	CaNP
Decomposition of cellulose, %	21.2	40.7	43.0	41.2	49.4	47.7

	Variants of experiment in trial II						
	Slag sand, coarse			Metallurgical		Hydrated	Lime
				lime	ground		
Ca applied, dt/ha	Control	57	114	57	114	57	114
Decomposition of cellulose, %	43.3	65.1	71.5	69.8	79.0	63.5	64.4

3. CO_2-release and decomposition of cellulose. The microorganisms by fertilization measures caused the intensified activity which manifests itself in an increased CO_2-production (Table 6). In both trials the CO_2-release increased depending on the increasing number of bacteria.

The decomposition of cellulose was determined by applying the gauze bag test. In trial I the bags were kept in the soil for seven months, and in trial II for five months (Table 7).

In both trials the cellulose was more completely decomposed in treated plots than the untreated ones. However, significant differences between the fertilization variants could not be ascertained.

4. Species composition of microscopic soil fungi and bacteria. The more often occurring species of microscopic soil fungi are presented in Table 8 for trial I.

Table 8

Species of microscopic soil fungi in trial I in the F-horizon, Population densities 1—5 (1 < 5)

Species of microscopic fungi	Variants of experiments		
	Control	Ca	CaNP
I. PHYCOMYCETES, MUCORALES			
Mucor ramannianus	5	4	4
Mucor, ser. Racemosus	2	1	1
Mucor, ser. Hiemalis	1		
Rhizopus nigricans	1	3	2
II. FUNGI IMPERFECTI, MONILIALES			
a) *Moniliaceae*			
Trichoderma köningi	3	4	3
Trichoderma lignorum	2	3	2
Penicillium ser. Monoverticillata I	3	2	1
Penicillium ser. Monoverticillata II	3	3	1
Penicillium ser. Monoverticillata III	2	1	1
Penicillium ser. Asimetria	2	1	
Penicillium ser. Biverticillata I	3	1	1
Penicillium ser. Biverticillata II	4	2	2
Verticillium sp. I	2		
Verticillium sp. II	1		
b) *Dematiaceae*			
Pullularia pullulans	1	2	2
III. MYCELIA STERILIA			
sp. 1	1	1	
sp. 2		1	
sp. 3	1	1	2

Moreover, there was a number of species which occurred only singly. The predominant number of fungus species was identical in all the three variants. The species occurring mostly are *Mucor*, *Penicillium* and *Trichoderma*. The frequency of the individual species was subject to changes in the different variants. To give an example, owing to fertilization with lime as well as CaNP, the number of *Mucor* species decreased slightly in comparison with the untreated variant.

In the untreated plots most *Penicillium* and *Verticillium* occurred in greater numbers than on treated plots. Altogether, fertilization caused some changes in the species spectrum of the microscopic soil fungi; however, the differences were not very large.

Fertilization led also to changes in the species spectrum of bacteria. The number of regularly occurring species was very low in the untreated raw humus. Only seven bacteria species forming no spores could be found. Owing to fertilization with Ca and CaNP the species spectrum was extended to five spore-forming species and 18 non-spore-forming species.

DISCUSSION

The results of the two fertilization trials show that the microbial activity in raw humus can be increased by appropriate fertilization treatments. On the poor site with extremely low P content in the eastern part of the Ore Mountains (Erzgebirge) an effect was obtained not only by liming, but also to a great extent by fertilization with Thomas phosphate. On the other hand, one year after N-fertilization the application of calcium ammonium nitrate without preliminary liming led only to slight changes. The lime fertilization experiment carried out in the Thuringian Forest showed the possibility of increasing the microbial activity even under extreme site conditions. In this work the differences between the individual lime forms were arranged in the following gradation: slag sand (coarse), metallurgical lime (ground), hydrated lime. In most cases the double lime quantity had a stronger effect than the single dosage, but the differences between the lime quantities were less pronounced than those between the lime forms applied. The more intensive decomposition of the raw humus and the nutrient release usually associated with it will have a positive effect on the nutrition of spruce under proper climatic conditions on edaphically poor sites. For trial I yield investigations were initiated. It is by no means the case that increased microbial activity must always be followed by an increase in yield. The relatively small quantity of mineral nitrogen can first temporarily be fixed by the increased number of microorganisms (see trial II, Table 2), so that at first the trees are supplied with soluble nitrogen in quantities smaller than prior to fertilization. For this reason great importance is attached to finding out the suitable fertilizer combinations.

The microbiological results obtained on a small number of sites cannot be applied to other site conditions without difficulty.

In these two trials the species spectrum of the microscopic soil fungi differed on the untreated plots; moreover, in trial II the bacteria were represented in numbers still smaller than in trial I. Therefore, it is expe-

dient to carry out microbiological investigations of soils in scientifically defined site units the so-called basic units, additionally taking into consideration the effect of tree species. At the same time, good prospects are thus given for generalizing the results.

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THEORETICAL ASPECTS ON THE PERSISTENCE OF ORGANIC MATTER IN SOILS

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The soil may be considered as an open system constantly changing energy and substances with the environment. The composition of the substrate influencing the amount of organic components such as the transformation of their living to nonliving forms, the change between high molecular and low molecular, humified and non-humified, chelated and non-chelated compounds should be particularly followed.

Intensified plant growth causes a more extensive exchange of energy and substances between soils and their environment. It seems to be very important to determine clearly the fluxes of energy and matter under various steady states of the system. Greater attention must be paid than before to the molecular chemical relations between humified and non-humified soil components.

Such investigations are most valuable because the soil conditions and the application of chemicals in agriculture are closely connected. The main essence of the problem seems to be the molecular properties which are responsible for the resistance of humic substances observed also in microbiologically active soils. The result of resistance of humic substances is a sojourn time from 10^3 years and a 10—100 fold enrichment from the yearly deposited organic residues. The presented study tries to find a qualitative answer to this question.

First of all the basis of microbial transformation of the so-called readily decomposible substances will be discussed. So far these substances are water soluble it is not difficult to understand their utilization by microorganims, because a diffusibility and a permeability through the cell membranes of microorganisms can be supposed. But there are easy decomposible not water soluble substances with high molecular weight too. A microbial action outside the membrane must certainly be assumed by such substances. Cellulose, chitin, keratin, amylose and other compounds may be regarded as substances of this nature.

Their microbial transformation begins with an ectoenzymatic depolymerization to soluble products with more favourable properties from the point of view of permeability. The way in which microorganisms can get information about the quality of the available, but in water insoluble matter must be omitted in the first approximation. This information is supposed to be responsible for the production of an enzymatic system suitable for the depolymerization of specific substrates.

Furthermore, we are not sure whether this enzymatic activity occurs in a contact effect regulated by the membrane of cells of microorganisms, or

it appears as a distant effect outside such a regulation. It can be assumed that enzyme production possessing molecular weights from 10^4 to 10^6 means metabolic expenditure requiring an economic use on the basis of contact effect and with the possibility of resorption.

On the one hand a certain minimum concentration is required by this actions justifying the expenditure of production of enzymes and on the other hand corresponding possibilities for enzyme-substrate complex formation postulated by Michaelis and Menten are required. These above mentioned suppositions are undoubtedly succeeded by biopolymers as starch, cellulose and proteins. In this case the process of depolymerization begins at places by sterically not protected macro-molecules. Under these conditions the yields of enzymatic actions can be expected to be high and the measure of transformation great because a high concentration of substrate and a strong succession of splitted bonds exist in the system. This is illustrated in Fig. 1, from which the feature of chemical forms can be seen as well as a schematic picture particularly demonstrating the continuous unlimited succession of the chemical bonds in case of starch, cellulose and protein serving as substrates by enzymatic depolymerization. This rule is to be considered as the important factor explaining the weak persistence of these substrates in the soil. In addition to this, it can be remarked that the designation of bonds are quite arbitrarily attached.

With regard to the so-called slow or difficult-to-decompose substances such as lignin which is at the same time a prototype of these substances we can ask the question which factors delay the decomposition? This phenomenon cannot be explained for sure because lignin is rich in low energy bonds C—C and C—O—C and at the same time is difficult to hydrolyze.

By clarifying the biochemical transformations occurring within the organism, the possibility of linkage or breakdown of these bonds without any difficulty by enzymatic catalysis is created.

It should be regarded that there is a three dimensional linkage of C—C and C—O—C bonds in lignin. The transformation of such units into low molecular units presumes an interaction of at least three enzyme systems from different kinds. Beside them quite various linking patterns exist in the side chains of these compounds (Freudenberg 1964). The figure shows only one of these various possibilities. Enzymatic attacks of the aliphatic structure occurring during the decomposition are therefore of a smaller importance. It should also be seen that enzymatic effect in the decomposition of lignin is relatively inhibited. This phenomenon can be considered as the main cause of the delayed decomposition of lignin molecules. In this concept it is very important that Flaig and Haider (1968) have found that the splitting of aromatic rings is the beginning of the microbial decomposition of lignin. But the ring system is the most fundamental basis of the lignin structure and therefore the microbial adaptation of this part of the whole structure must be supposed as a most probable success. This result is an indirect confirmation of the whole view of this study. In this concept it must be stated that the macromolecular aromatic linkage of humic substances is not responsible for their long-term stability in the soil. Moreover, various ways of reactions must be considered as possibilities leading to transformation of humic substances and a lot of compounds may be transformed to humic substances or may react with them.

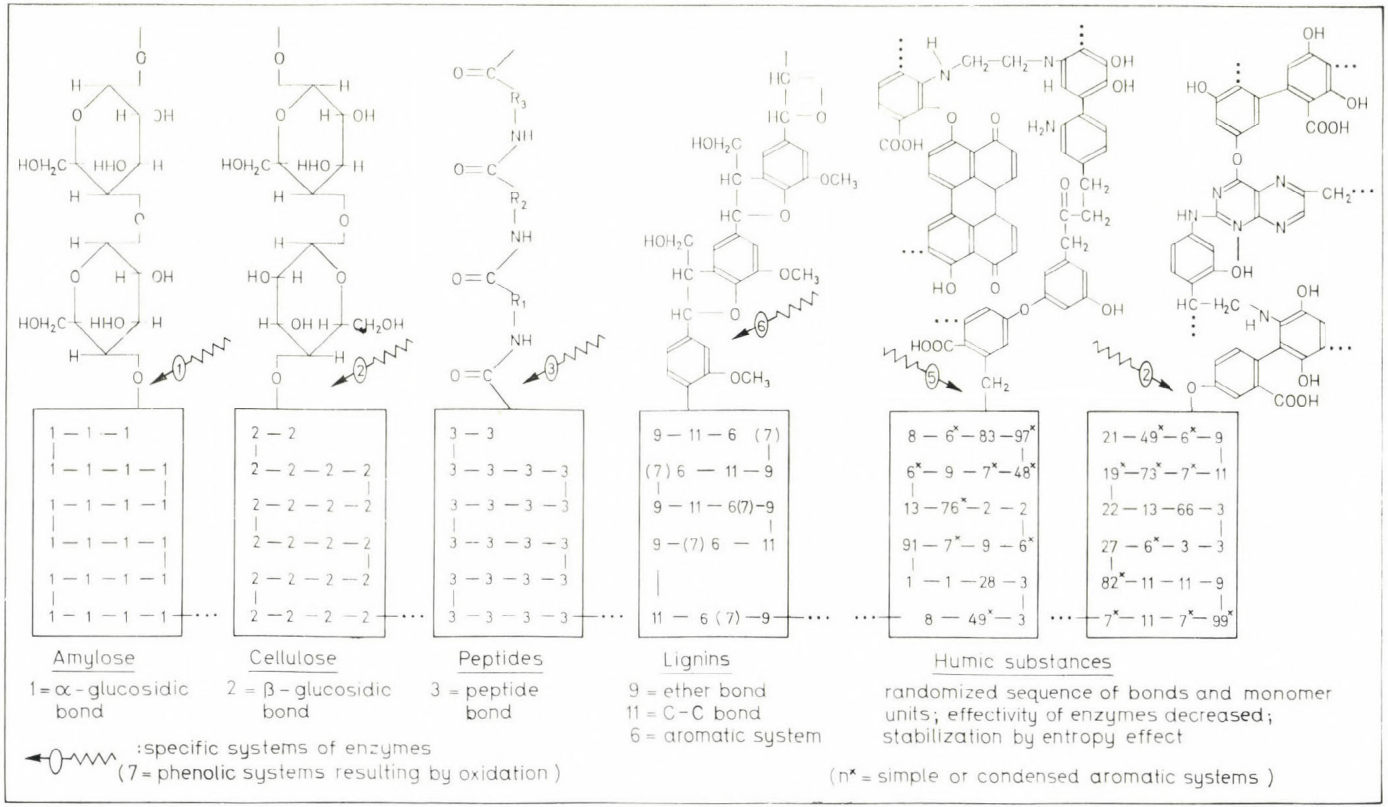

Fig. 1. The enzymatic depolymerization of natural polymers under the aspect of the state of molecular order

With regard to this it is to be postulated that there is a state of molecular order of humic substances whose final stage is marked by absolute irregularity and nonreproducibility. In conclusion, the high stability of humic substances in soils with a great microbial activity can be explained. Attack of enzymes is always specific and the exceptional speciality of humic molecules results in an enzymatic activity against the humic substances because the enzymes are not able to use these compounds as substrates (see also Kleinhempel 1969). Figure 1 shows this concept. Two types of humic molecule parts represented in the figure should be in agreement with the present stage of our knowledge on this subject.

The simplified scheme moves out from the concept that humic acids consist of a practical unlimited number of monomer units which are linked by nearly 100 various linkage sequences. The type of chain structure is characterized by the linkage atoms and their neighbouring atoms.

The possibility of variations between the reacting compounds and their linkage types leads to a great variability in this humus structure. The reactivity and frequency of aromatic ring systems are responsible for their accumulation in the structure of this permanently changing system. The figure shows that a supposed attack yields results unlikely to realise any enzymatic reactions which could be characterized in a matrix with statistical distribution of the substrates. Regarding this concept and assumed conditions in which $\Delta G = \Delta H - T\Delta S$ is evaluated as a form for processes starting spontaneously in formation of humic substances not so very favoured by high values of enthalpy of free set reaction (negative ΔH values) but more by a high income of entropy (positive ΔH values).

Humic substances appear under these aspects as thermodynamically very stabilized and highly randomized molecular structures whose enzymatic depolymerization is loaded with a high grade of improbability.

Furthermore, high values of activation entropy can be expected by the three-dimensional linking of humic macromolecules which decreases the possibilities of a favourable arrangement of the enzyme-substrate-complex.

At last humic substances with a greater maturity have entropy maximum which cannot be exceeded by a destruction of the structure to free diffusible monomer units.

Thereby the reception microorganisms and the mineralization is naturally blocked. Beside them a spontaneously total reaction with positive values of entropy and negative values with enthalpy should be added.

At the same time by means of the theory given here it is possible to explain why certain nonhumic substances e.g. proteins and celluloses are always associated with humic substances and thereby protected against the activity of microorganisms (Mayaudon 1968). The matrix of humic substances has here a function as adsorbent on the strength of their randomized molecular structure can give a steric protection against enzymatic influences.

The "priming effect" will be also accessible to a thermodynamical interpretation. One has to go out from the fact that the level of enzymes increases on application of easily decomposable substances resulting in a higher possibility of collision of enzyme and substrate on the humic matter.

Under such conditions the grade of improbability for the degradation is decreased. The same mechanism can be taken as basis for the "Birch effect".

The entropy stabilization "humification" of organic matter in the soil is a permanent process which is favoured by sorption and gel permeation of nonhumified materials on and into the humic substances respectively, by local pH changes in microscopical areas, by swelling, shrinking, intramolecular change and by pigment reduction of microorganisms. On resting in microbial activated soils the probability increases that these processes may have a stabilization effect and that the humification coefficient rises from 0.2 - 0.3 in case of nonhumified materials to 0.99 in the humified state. The longer the rest period needed for stabilization, the greater the losses caused by mineralization and perhaps mainly the natural level of humic substances is low.

The randomized molecular structure confirmed as the essential aspects of the state of humic matter, possibilities ought also to be found which may reduce the required time for the formation of humic substances and the rest period of humic substances influencing the stability and level of humic matter.

SUMMARY

1. The degradation of polymer substances in soils has been considered particularly from the point of view of the depolymerization as the first step of accelerated degradation reaction.
2. In comparison with amylose, cellulose, protein, the depolymerization of lignin is more difficult while cooperating systems theoretically are to be supposed free diffusible monomer units. Thereby, the natural way of lignin degradation probably occurs over the splitting up to the frequent and regular aromatic structures in the lignin molecule.
3. Humic substances are natural organic polymers with the highest grade of molecular irregularity or with the highest structure of entropy. These humic substances are stabilized against microbial activities and a collision of enzymes and the substrate is most unlikely.
4. Absorbed nonhumic substances may also be stabilized by this mechanism.

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ROLE OF MICROORGANISMS IN THE TRANSFORMATION OF HUMIC SUBSTANCES OF CAUSTOBIOLYTES

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In connection with the fact that agriculture endures deficiencies in organic fertilizers, a problem arises of the search for new sources of primary products which could be used for obtaining the effective fertilizer. Therefore, attention should be paid to the application of caustobiolytes (peat, wastes of brown coal), containing a large percentage of humic substances. On the basis of these components the method for preparing the biomineral fertilizer was developed. The main principle in obtaining this fertilizer is to control the intensification of the microbiological processes in the mixture of caustobiolytes with wastes of the chemical, metallurgical and food "industries". The biomineral fertilizers are black, dry masses with maximum moisture of 40—50% and contain the main plant nutrients (nitrogen, phosphorus, potassium) about 200 kg in 10 tons of the ready product. Besides the nutrients, they contain rich useful microflora. Regulation of the microbiological processes for maximum extraction of the nutrients from caustobiolytes and accumulation of the products as a result of physiological activity of microorganisms are the important links in the preparation of the biomineral fertilizers (BMF).

It should be noted that the data on grouping of microorganisms participating in mineralization of humic substances are inadequate. Winogradsky (1952) advanced a hypothesis concerning the existence of the specific group of microorganisms which take part in decomposition of humates, and called these microorganisms autochthonous microflora, Tepper (1969) succeeded in isolating the pure cultures of some representative of this group. He studied their morphological and physiological peculiarities and then established their generic and specific origin. The works of Mishustin, Nikitin and Ochilova (1960), Nikitin (1967), Smaly et al. (1970) and also of some other authors showed that transformation of the humic substances occurs under the effect of the saprophytic microorganisms as well. Addition of carbohydrates and soluble forms of nitrogen to the substrate under study considerably accelerates the decomposition process of the humic substances under the effect of different microorganisms and especially of bacteria from the genus *Pseudomonas*.

Caustobiolytes (peat, brown coal), being the main components of BMF, contain considerable amounts of humic substances. In their decomposition the microorganisms play an essential role.

Sodium humate was obtained from lowland peat and brown coal which decomposed on the gel plates under the effect of different microorganisms (Table 1). Sodium humate decomposed most actively under the effect of bacteria from the genus *Pseudomonas* as well as of other microorganisms.

Table 1

Number of zones of solution (sodium humate) per 1 cm² of gel plate (term of incubation — 60 days)

Microorganisms	Total number of zones of solution per 1 cm²	Number of zones of solution which are completely translucent
Pseudomonas sinuosa	89	63
Pseudomonas fluorescens	64	13
Bacillus glutinosus	77	12
Bacillus megatherium	47	6
Mycobacterium citreum	69	54
Actinomycetes I.	76	56
Azotobacter chroococcum	82	58
Trichoderma lignorum	57	23

Furthermore investigations were conducted as to the decomposition of sodium humate had some humic acids (humic, ulmic, lignofulvonic and fulvic acids) under the effect of microorganisms taking into account the carbon decrease and accumulation of the hydrolyzed and ammonia forms of nitrogen. The above mentioned humic acids were obtained from the lowland peat by Williams' method (1965). Under aseptic conditions the preparations of sodium humate or some humic acids, chalk as well as suspension of the microorganisms under study were introduced to 1 kg of the roasted river sand. The amount of carbon in the substrate under investigation was determined by the method of Tyurin, ammonia-nitrogen — after Nessleri-

Table 2

Quantitative changes of carbon, ammonium and hydrolyzed nitrogen resulting from humate decomposition by microorganisms (mg per 100 g of dry substrate)

Variants	At the beginning of the experiment			In 2 weeks			In 2 months		
	Carbon	NH_3 nitrogen	Hydrolyzed nitrogen	Carbon	NH_3 nitrogen	Hydrolyzed nitrogen	Carbon	NH_3 nitrogen	Hydrolyzed nitrogen
Control (sand, chalk, Na-humate)	940.0	2.9	7.1	953.0	2.9	—	891.0	2.7	3.5
Sand, chalk, Na-humate, *Pseudomonas sinuosa*	1155.0	3.8	6.6	1036.0	6.5	8.6	849.0	3.5	4.3
Sand, chalk, Na-humate, *Actinomycetes I.*	1184.0	3.2	6.8	1107.0	5.9	7.4	789.0	3.8	4.4
Sand, chalk, Na-humate, *Trichoderma lignorum*	1140.0	3.5	6.6	1117.0	6.3	9.0	955.0	3.8	4.0

zation of extracts – by colorimetry and hydrolyzed nitrogen according to Tyurin and Kononova (1963). The data obtained concerning decomposition of sodium humate are given in Table 2.

From the data presented above one can see that there is an increase in the amout of hydrolyzed nitrogen and more pronounced in the case of ammonia after two weeks. By two months of incubation the amount of these nitrogen-forms decreases as well as the carbon-content according to the microorganisms applied.

The data of humic acid's decomposition are given in Table 3. As in the previous experiment the amount of carbon decreases and that of

Table 3

Quantitative changes of carbon, ammonia and hydrolyzed nitrogen resulting from humic acid decomposition by microorganisms (in mg per 100 g of dry substrate)

Variants	At the beginning of the experiment			In 2 months			In 3 months		
	Carbon	NH_3	Hydrolyzed nitrogen	Carbon	NH_3	Hydrolyzed nitrogen	Carbon	NH_3	Hydrolyzed nitrogen
Control (sand, humic acid)	1881.75	3.16	5.72	1880.0	3.02	6.00	1880.9	3.01	5.5
Sand, humic acid, *Pseudomonas sinuosa*	1621.2	4.13	6.63	1436.4	7.34	10.88	1258.2	6.74	9.24
Sand, humic acid, *Mycobacterium citreum*	1698.4	3.74	6.02	1780.5	6.75	10.64	1363.7	6.74	8.80
Sand, humic acid, *Actinomycetes I.*	1910.7	2.50	6.35	1840.4	8.78	11.57	1424.0	6.41	9.08
Sand, humic acid, *Fusarium oxisporum*	1925.18	3.27	5.58	1720.7	7.82	11.39	1861.5	5.71	8.74

ammonia and hydrolyzed nitrogen increases. The analogous phenomenon was observed in experiments with decomposition of ulmic, lignofulvonic and fulvic acid as well. Carbon loss and accumulation of the mobile forms of nitrogen testify to the fact that the investigated microorganisms promote the decomposition of humic substances.

In Table 4, data are presented on accumulation of vitamins of B-group during the decomposition of humic acid. From the given data one can see that in a month from the beginning of the experiment the amount of biotin, pyroxidin and nicotinic acid increase in the substrate under study followed by decrease of their quantity. The presence of free amino acids was also taken into consideration with decomposition of humic acid in a month from the beginning of the experiment. The data of this experiment are given (Table 5). Under the effect of microorganisms their amount rises in most cases and amino acids are found which were not observed in the

Table 4

Accumulation of vitamins during decomposition (in μg per 100 g

Variants	Nicotinic Acid			
	At the beginning of the experiment	In 1 month	In 2 months	In 3 months
Control (sand, humic acid)	t r a c e			
Sand, humic acid, *Pseudomonas sinuosa*	1.05	10.96	3.64	2.47
Sand, humic acid, *Mycobacterium citreum*	1.13	22.27	0.97	1.48
Sand, humic acid, *Actinomycetes*	0.92	17.87	2.56	1.85
Sand, humic acid, fungus *Fusarium oxysporum*	0.86	17.32	2.59	2.97

material under study. Such free amino acids as tryptophan and glutamic acid were found in the medium of the experimental variant with the bacterial culture *Pseudomonas sinuosa*. Tyrosine, ornithine, lysine, glycine, valine, histidine, glutamine were observed in considerable amounts.

The data given in Tables 4 and 5 testify to the fact that with decomposition under the effect of microorganisms of humic substances the medium

Table 5

Presence of free amino acids during decomposition of humic acid by microorganisms (in μg per 1 g of dry weight of humic acid)

Amino acids	Variants of experiment				
	Control (sand, humic acid)	Sand, humic acid, *Pseudomonas sinuosa*	Sand, humic acid, *Mycobacterium citreum*	Sand, humic acid, *Actinomycetes I*	Sand, humic acid, fungus *Fusarium oxysporum*
Cystine	—	21.3	1.53	4.8	—
Arginine	—	1.92	—	—	81.6
Serin	1.81	15.5	11.2	687	8.2
Tyrosine	3.4	—	—	1.4	20.6
Ornithine	1.7	3.5	—	—	—
Asparaginic acid	—	—	—	8.7	7.8
Glutamic acid	—	14.4	—	—	—
Isoleucine	—	2.0	—	—	—
Lysine	3.4	28.2	2.6	37.4	—
Glycine	2.55	8.5	7.3	7.3	10.5
Alanine	—	68.0	27.2	4.3	—
Valine	6.8	—	—	1.5	10.2
Histidine	—	14.1	—	76.5	—
Glutamine	—	20.9	—	—	—
Tryptophan	—	25.5	—	—	—
Leucine	5.3	2.0	—	—	—

of humic acid by microorganisms of drysubstrate)

Pyridoxine				Biotin			
At the beginning of the experiment	In 1 month	In 2 months	In 3 months	At the beginning of the experiment	In 1 month	In 2 months	In 3 months
t r a c e				t r a c e			
0.54	0.78	0.024	tr.	tr.	0.45	0.33	0.27
0.59	1.28	0.021	tr.	tr.	0.32	0.29	0.23
1.43	1.71	0.025	tr.	tr.	0.49	0.35	0.19
0.4	0.9	0.025	tr.	tr.	0.39	0.34	0.29

becomes enriched in the biologically active substances which are of great importance for increasing the quality of the biomineral fertilizer.

The preparation of the biomineral fertilizer under industrial conditions, intensification of the microbiological processes in this fertilizer was realized by selection of the corresponding components and introduction of the microbial enzymes of two bacterial cultures — *Pseudomonas sinuosa* and *Bacillus megatherium*.

Under conditions of the Kiev plant for bacterial preparations, a technology was developed for preparing the mother culture in the form of a dry preparation.

The second culture was used as the bacterial fertilizer for the preparation of phosphorobacterin.

In the places of BMF preparation in the local institutes of "Selkhoztekhnika" the microbial enzyme was prepared from the mother cultures which was used, as a component of BMF. The investigations showed that in the process of BMF maturity the bacterial cultures of enzymes develop well in the fertilizer and with its introduction into the soil they become acclimatised to the rhizosphere of agricultural plants.

The investigations showed as well, that accumulation of the biologically active substances in the fertilizer and in the soil occurs both with preparation of BMF under industrial conditions and with its introduction into the soil.

SUMMARY

1. Sodium humate is obtained from peat and wastes of brown coal and from lowland peat — humic, ulmic, fulvic- and lignofulvonic acids which were investigated as to their ability to decompose under the effect of microorganisms.

2. With decomposition of humic acids, carbon loss and accumulation of ammonia and hydrolyzed forms of nitrogen were observed.

3. With decomposition of the humic substances by microorganism accumulation of the biologically active substances — vitamins of B group and amino acids occurred in the substrate.

4. The bacterial cultures were selected and the technology of preparing the mother culture for production of BMF under industrial conditions was developed.

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INFLUENCE OF ORGANIC MATTER ON THE HETEROTROPHIC BACTERIA IN DIFFERENT SOILS

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The soil contains a considerable number of microorganism species whose functions and special importance for the decomposition of organic matters are insufficiently known so far. Moreover it is hardly known whether several microorganisms are responsible for the decomposition of organic matter in the different soils.

The current investigation tended to study which microorganisms actually perform the decomposition of determined organic substances in the different soils. For this it was necessary, first, to carry out a qualitative analysis of the microflora and consequently to study particularly the physiological performances and potentialities of the predominating microorganisms.

METHOD

For the present-day level of microbiological reseach it is appropriate to maintain unchanged conditions throughout the laboratory investigations; in field investigations there are too many variable factors affecting the development of microorganisms. In model tests different soil types were incubated at constant temperatures and optimal soil moisture, the incubation period ranged from several months up to 2 years. Specific organic substances have been used, as energy sources as carbohydrates: glucose, maltose, pectin and cellulose and the proteins: peptone, casein, casein hydrolyzates and gelatin, separately and in several mixtures.

The microflora was quantitatively and qualitatively investigated by applying the dilution-plate-method after Koch. Heterotrophic soil microorganisms, growing on collective culture media, were subjected to the examination. Bacteria, actinomycetes and fungi respectively were separately studied on suitable medium.

The evaluation of the dilution plates has been made according to the type method in which by preliminary studies the colonies mainly existing on the petri-dishes are fixed as types after their integration in a systemic unity has been ensured.

RESULTS

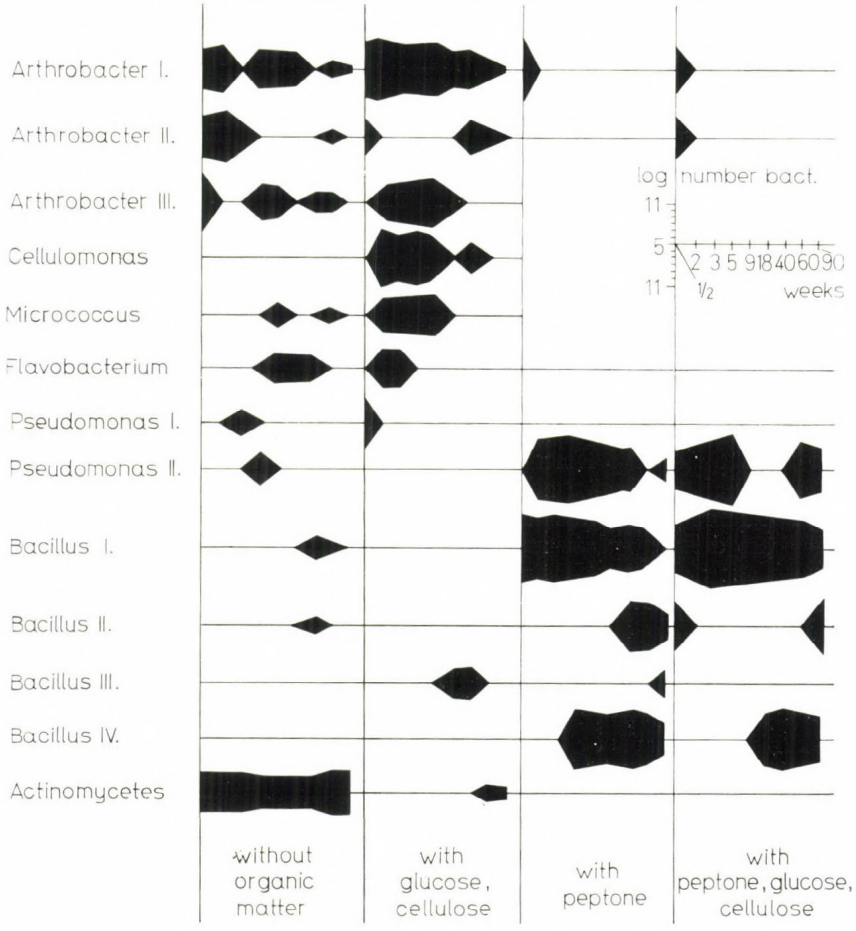
In arable soils the decomposition is mainly brought about by bacteria, whereas fungi and actinomycetes play a less important role in the mineralization of readily decomposed organic matter.

Organic manure produces a definite microflora which is primarily affected by the respective organic substance. The different soils, however, are in position to increase several species of certain physiological and systematic groups respectively.

Owing to the importance of bacteria we will demonstrate the development of different types in this group. Choosing sandy soil (Fig. 1) as an example we desired to demonstrate the change in the bacterial flora after the addition of carbohydrates and protein, in this special case glucose and cellulose (G, C) and peptone (P). In the graph the height of the numbers of microorganisms has been plotted both upwards and downwards on the ordinate in a logarithmic scale. From the sizes of the figure the predominating microorganisms throughout the investigation period can be clearly distinguished.

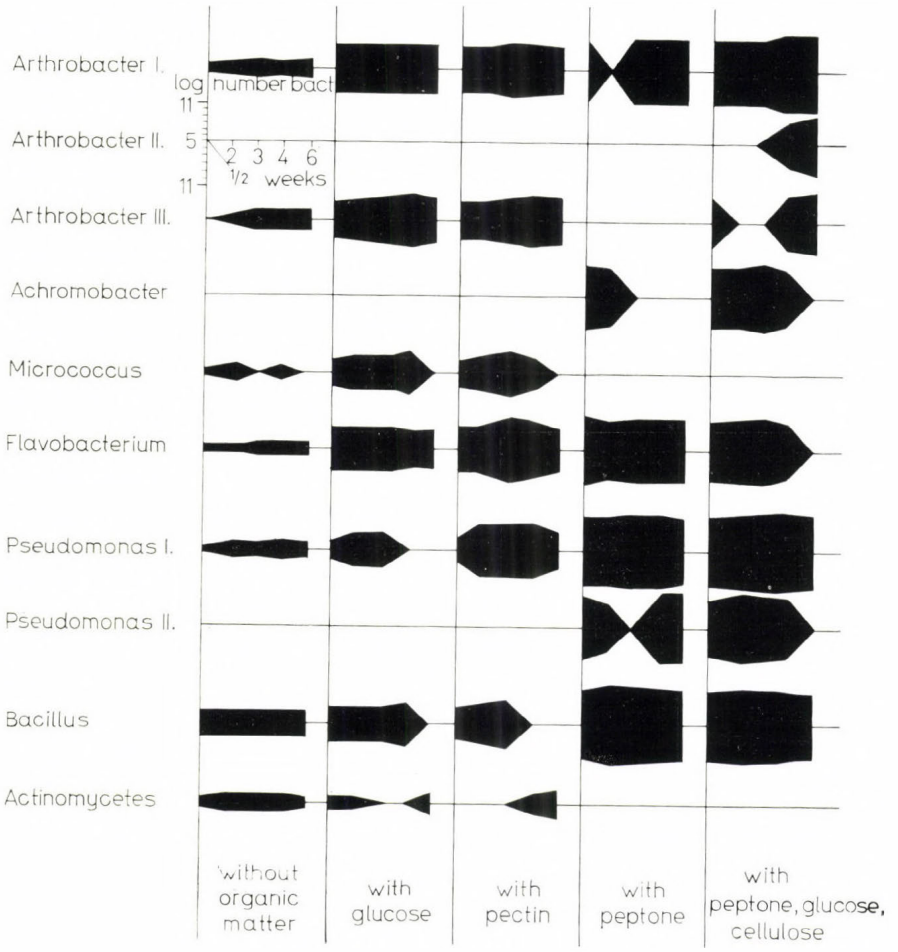
Out of the basic flora of the unmanured soil, characteristic groups developed: adding glucose and cellulose preferable coryneforme bacteria multiply such as *Arthrobacter-*, *Cellulomonas-* and *Mycococcus*-species. Adding

Fig. 1. Change of the bacterium flora in sandy soils by organic manuring



peptone, however, *Bacillus*-species are stimulated along with proteolytically active *Pseudomonas*. Conversely, coryneforme bacteria are depressed if peptone is used. Also *Bacillus* and *Pseudomonas* decrease with glucose and cellulose. If a complex manuring consisting of peptone, glucose and cellulose is applied the influence of peptone is decisive to such a degree that the bacterial flora develops in the same way as the variant of peptone. *Actinomycetes*, which dominate in unmanured soil decrease strongly after manuring with organic matter, especially, if protein is used. Only after a longer period, about 3 quarters of a year, after the addition of organic matter, they start developing again. This trend applies to all carbohydrates and to all proteins. Thus the flora was more or less identical after the addition of monosaccharides, disaccharides or polysaccharides. As a matter of fact, it was surprising to notice that at an addition of pectin and especially of cellulose a flora appears which resembles a flora brought about

Fig. 2. Change of the bacterium flora in an organic soil by manuring



by a glucose additive. Since only definite flora of microorganisms is able to develop on the culture media these findings will meet with a more ready comprehension.

Most of the detected microorganisms therefore will live on secondary decomposition products in the "intermediate phase" of organic matter. So they can be reckoned to the flora of carbohydrate-manured soils. They must be understood as "follow up organisms" which are equally responsible for the overall decomposition of the macromolecular carbohydrates, even though they are not in a position to develop typical pectinases or cellulases.

Generally these trends of bacteria are to be observed in all mineral top soils. In organic soils, however, this tendency is not so clearly demonstrable (Fig. 2). Also out of a basic flora (variant without organic matter) the stimulation of certain groups takes place; so a stimulation of *Bacilli* in the protein-variants and a stimulation of *Arthrobacter* in carbohydrate-variants. But also it is to be seen that *Arthrobacter* are promoted in peptone manured soils and *Bacilli* multiply at a faster rate with carbohydrates.

Furthermore the species belonging to the genera *Micrococcus*, *Flavobacterium* and *Achromobacter* increase more rapidly too. As the admixture of 1% of organic matter is fairly low in proportion to the total amount of organic matter contained in the fen soil (50%), the manifold flora is clearly understood. It is actually amazing that 1% of this added matter affects such a marked change of flora for a longer time interval. This demonstrates the importance of freshly added organic matter for the development of the microflora.

The causes for a rapid multiplication of definite bacteria species are manifold. Their enzymatic potentialities, also the changes in the soil reac-

Table 1

Biochemical activity of different soil bacteria

	Taxonomic group[1]	Gelatin liquefaction	Casein hydrolyzis	Growth on nitrate-agar
Bacillus I	Megatherium	+++	+++	−
	Cereus	+++	+++	−
	Mycoides	+++	+++	−
Bacillus II	Subtilis	+++	+++	+
Bacillus III	Circulans	++	−	−
	Licheniformis	++	+	±
Bacillus IV	Lentus	−	−	−
Pseudomonas I	Fluorescens	−	±	++
from sand soil				
from fen soil	Fluorescens	++	++	++
Pseudomonas II		+++	+++	++
Achromobacter	−	+	+	n. b.
Arthrobacter I	Oxydans	−	−	+++
Arthrobacter II	Globiforme	−	−	+++
Arthrobacter III	Aurescens	±	+	+++
Brevibacterium	−	+++	+++	+

1 The type is closely related to the designed group.

tion and the antibiotic environment are important factors. Many *Bacilli* show a strong proteolytic activity. This also applies to some *Pseudomonas*. By contrast, most of the isolated coryneforme bacteria had no or little proteolytic power (Table 1). Thus the stimulation of *Bacilli* and *Pseudomonas* in protein-manured soils as compared with the *Arthrobacter* becomes more readily comprehensible: *Arthrobacter* tend to lag behind in the competition for nutrition. In this context it must be recalled, furthermore, that proteins after being broken up are able to diffuse into the cells and act here after desamination as sources of energy.

Bacillus lentus (Bac. IV) is an exception to the rule, among the *Bacilli*. It is unable to break up gelatin and casein and so it is clear why we fail to detect Bac. IV at the beginning of the incubation period. Only later it can be found, after hydrolyzing protein by other organisms. Since proteolytic enzymes act also as exoenzymes more proteins are broken up than can be assimilated by the bacteria themselves; hence, a sufficient quantity of N-rich organic matter can be utilized by the *Bacillus lentus* group.

On the other hand the *Arthrobacter* are superior to the *Bacilli*, if organic nitrogen is not available in sufficient quantity. Failure of some soil *Bacilli* to synthesize particular aminoacids may be the underlying cause. This became evident at growth trials conducted with deficient media (Table 2).

Table 2

The growth of different bacteria in deficient-media
(Vagnerova et al., 1960)

	Number of tribes	Basic medium	Basic medium + amino acids	Basic + amino acids + yeast extract
Bacillus I	12	2	11	12
Bacillus II	6	4	6	6
Bacillus III	6	1	6	6
Bacillus IV	8	0	7	8
Pseudomonas I	7	7	7	7
Pseudomonas II	10	9	9	10
Arthrobacter I	10	10	10	10
Arthrobacter II	8	8	8	8

Thus, if we choose the *Bacillus* I-group as an example, only 2 out of 12 succeed in growing in a basal medium without amino acids. On the other hand, all *Arthrobacter* strains examined are capable of synthesizing all needed amino acids, vitamins and other factors of growth since all of them have been growing in a basal medium.

As an example of the influence of the pH-values the behaviour of the *Bacillus cereus-megatherium*-group (Bac. I) and the *Bacillus lentus*-group (Bac. IV) will be shown. After addition of peptone a different development takes place in the same sandy soil, depending on addition of $CaCO_3$ and resulting in various pH-values (Fig. 3): In the limed soil mainly *Bacillus I* developed, in unlimed soils *Bacillus IV*.

The examination of various soils has shown that the manuring with organic matter developed specific microorganisms, which can be classified

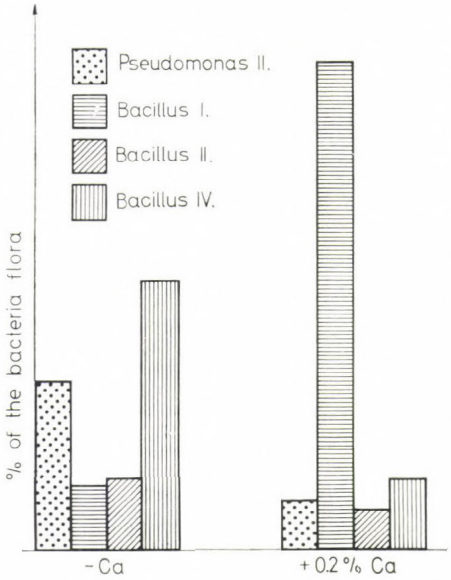


Fig. 3. The influence of the soil reaction on the development of different bacteria after manuring with peptone. Starting pH: (unlimed soil) 4.8, (limed soil, 6.8. Maximum pH: (unlimed soil) 7.5, (limed soil) 8.2 after deamination

within physiological production groups. In the case of these investigations they are integrated into the carbohydrate and protein-decomposing group. If organic matter is used as a manurial agent it is the absence or the presence of proteins that determined the development of one group or the other. By transforming inorganic nitrogen into the organically bound state, carbohydrate, manuring may trigger off successions and at a later stage of decomposition it may cause microorganisms to develop equally belonging to the protein-decomposing group. If carbohydrates are not supplemented with nitrogen not enough protein containing matter can be produced and the carbohydrate-decomposing flora is preserved for a longer period.

In conclusion it can be stated that out of the numerous microorganisms, being often described as soil inhabitants and being often characterized by a manifold enzymatic potence in decomposing organic matter, only a relatively limited number was found to decompose organic matter in the soil. These microorganisms belong only to few genera. Among bacteria particularly coryneforme bacteria along with species of *Bacilli* and *Pseudomonas* are to be found in all soil types if organic matter has been applied before. In addition also *Achromobacter-*, *Flavobacterium-*, *Micrococcus-* and *Brevibacterium*-species are enabled to multiply rapidly; yet they cannot be found in all soils. On the basis of the present investigations we cannot subscribe to the frequently repeated opinion that in the individual soils a fairly varying flora may account for the decomposition of organic matter.

Moreover, the opinion that on addition of various organic matter to the soil an extremely diverse microflora will come forth can only be endorsed with all due reservation, especially if more complex organic matter is concerned. On comparing the microflora of soils manured with alfalfa- and grass meal no essential qualitative differences have been found. There was merely a slightly higher percentage of *Bacillus-*, proteolytically active *Pseudomonas-* and *Brevibacterium*-species as far as the manuring with alfalfa meal is concerned, this must be ascribed to the higher protein content of alfalfa meal.

We are of the opinion that the soil with its 3 phase systems, its water content and its porosity despite certain textural and structural differences and differences with regard to organic matter content actually constitutes a characteristic and selective locus allowing only few organisms, being cer-

tainly well adapted to this locus, to multiply and to utilize completely the existing nutritive substances.

The usually aerobic conditions in the soil constitute an important selecting factor. Microorganisms possessing no complete enzyme chains for oxidative breaking up of the organic compounds will be at a disadvantage. All promoted microorganisms isolated throughout the tests betrayed a strong cytochromoxidase activity. Thus they are able to oxidize organic matter up to oxygen and to utilize the nutritive substances efficiently resulting in a substantial yield of energy.

SUMMARY

Definite organic substances have been examined since the influence on the variously composed microflora could only be detected in this way.

Different carbohydrates and proteins were subjected to investigation in model tests. In arable soils the decomposition is mainly brought about by bacteria, whereas fungi and *Actinomycetes* play a less important role in the mineralization of readily decomposable organic matter.

Organic manure produces a definite microflora which is primarily affected by the respective organic substance. The different soils, however, are in a position to increase several species of certain physiological or systematic groups respectively.

If protein is used as a manure, *Bacillus* and *Pseudomonas* develop above all, but also *Achromobacter-* and *Brevibacterium*-species can propagate in some soils. By admixing carbohydrates into the soil the propagation of coryneforme bacteria is favoured.

The promotion of *Arthrobacter*-species by compounds which are poor in protein is dependant on its ability to easily assimilate inorganic nitrogen. Thus they have an advantage over a large number of *Pseudomonas* and first of all *Bacilli* requiring essential amino acids for their growth. The development of *Bacilli* can only take place if a sufficient quantity of protein substances as a nutritional source has been synthesized by means of other organisms.

Generally the decomposition of organic matter in soils is brought about only by a relatively limited spectrum of microorganism species which are particularly adapted to the soil conditions.

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EFFECT OF ORGANIC MATTER, AERATION AND BENTONITE ON MICROORGANISMS AND PLANTS

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Clay minerals can form complex bonds with organic compounds thus protecting them against decomposition (Lynch and Cotnoir, 1956, Pinck et al. 1954). According to several authors, clay minerals can stimulate or inhibit soil microorganisms (Zvyagintsev 1959, Esterman and McLaren 1959, Macura and Pavel 1959, Novaková 1966, Stotzky and Rem 1966). Bentonite stimulated their development in most of the cases. It is possible to expect that bentonite will also influence the decomposition of organic matter in the soil as well as the quantity and quality of microbial metabolites. In this respect, aeration may have the same sort of influence. Vang (1967) discovered a large amount of organic acids in excessively moist soil. Organic acids (Vang 1967), amino acids (Kubista 1965) and other metabolites and components of post-harvest residues may develop an influence on the growth of the plants.

This work deals with the influence of bentonite and of the matter of plant origin on microbes and plants under various degree of aeration.

MATERIALS AND METHODS

Pure quartz sand was mixed with 5% lucerne meal and 1% separated bentonite. The control sample was without bentonite. The mixture was moistened and enriched with nutrient salts of the Knop solution; it was inoculated with soil suspension. This substrate was put in glass containers with covers and a half of the samples was aerated. The incubation temperature was 30 °C and the moisture was set to 60% of maximum water capacity.

Similarly, an experiment was conducted with degraded chernozem (silty clay, according to mechanical analysis). The mixture was moistened only with distilled water.

The dynamics of the development of microorganisms was determined on the count basis by means of the agar-plate method; Thornton's medium and meat-peptone agar were used for the bacteria, Thornton's and starch media for *Actinomycetes* and Martin's medium was used for the counting of microscopic fungi.

The influence of water extracts of the particular mixture was determined according to the root length of lettuce seedlings which were cultivated in petri-dishes on filter paper moistened with sterile extract. The extracts were obtained by mixing the substrate with distilled water (1 : 1) which was followed by filtration.

RESULTS AND DISCUSSION

Results show a stimulating effect of bentonite on the multiplication of microorganisms decomposing lucerne meal. This effect was more evident in the non-aerated samples since the quantity of microorganisms in the aerated samples was influenced decisively by the aeration itself.

Sand Samples. As can be analysed from the data in Fig. 1, the multiplication of bacteria in the aerated variants started at the very beginning of the experiment and reached the maximum very quickly. In the non-aerated variants their quantity dropped at the beginning of the experiment and the maximum was reached on the 28th day. The initial drop is evidently linked with the inevitable adaptation of the microflora to the oxygen-poor environmental conditions. Although bentonite stimulated the non-aerated-sample bacteria for 14 days, the non-aerated variant was under the stimulative effect for the whole experimental period.

Similar course of development could be observed (Fig. 2) with bacteria short of organic nitrogenous compounds. The difference was, in the first place, in the slower development of the initial stage of the aerated variant. On the other hand, the non-aerated variant showed faster multiplication and more expressive effect of bentonite.

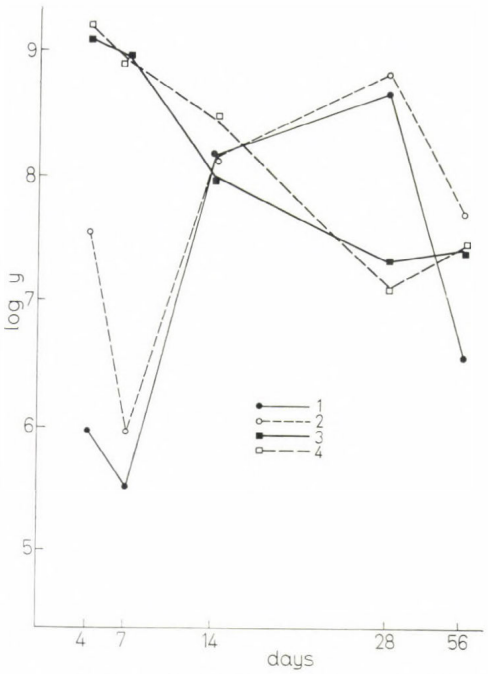


Fig. 1. Number of bacteria developed on Thornton's media; sandy variants. 1 = non-aerated: without bentonite, 2 = non-aerated: with 1% bentonite, 3 = aerated: without bentonite, 4 = aerated: with 1% bentonite, u = number of living cells

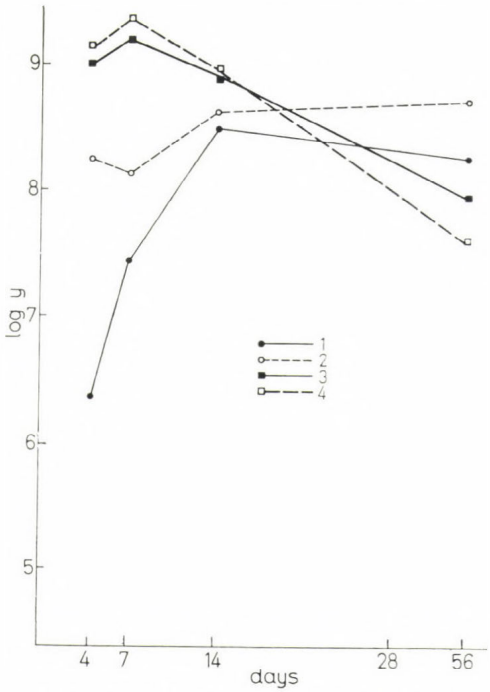


Fig. 2. Number of bacteria developed on meat-peptone agar; sandy variants. Legend: same as for Fig. 1

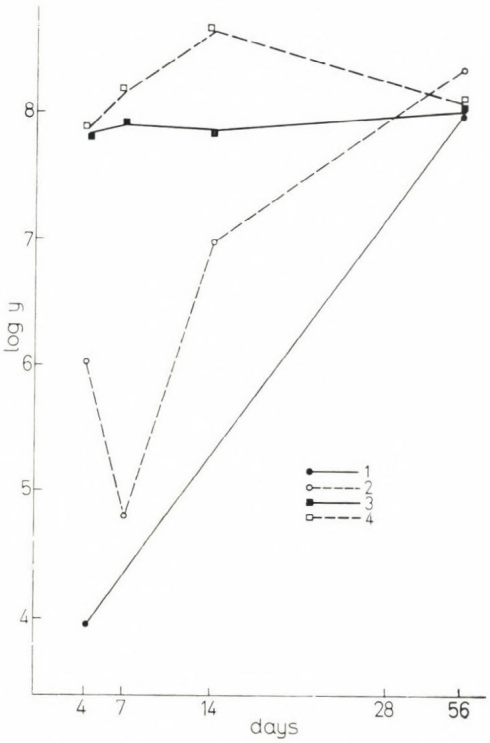


Fig. 3. Number of spore-forming bacteria on meat-peptone agar; sandy variants. Legend: the same as for Fig. 1

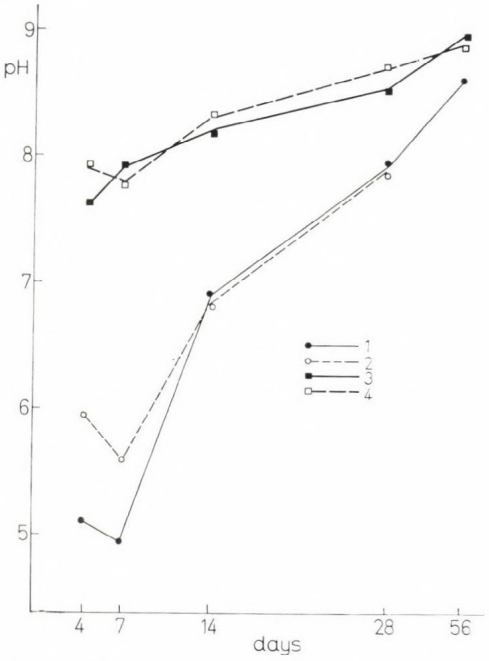


Fig. 4. Change of pH; sandy variants. Legend: the same as for Fig. 1

Fig. 5. Number of actinomycetes; sandy variants. Legend: the same as for Fig. 1

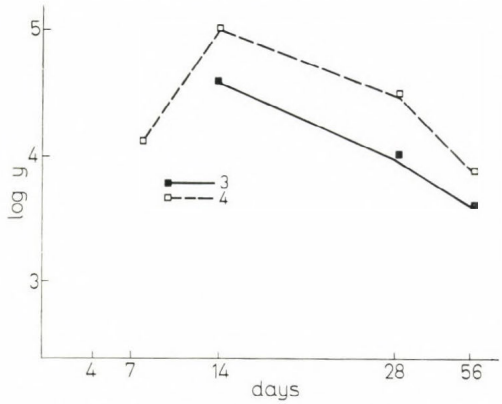
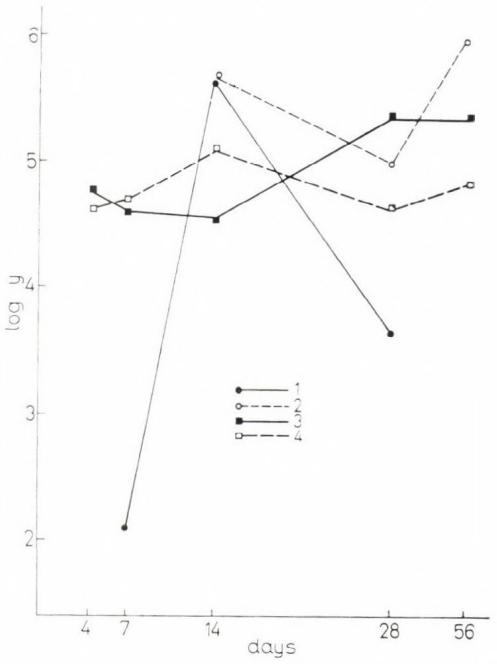


Fig. 6. Number of fungi; sandy variants. Legend: the same as for Fig. 1



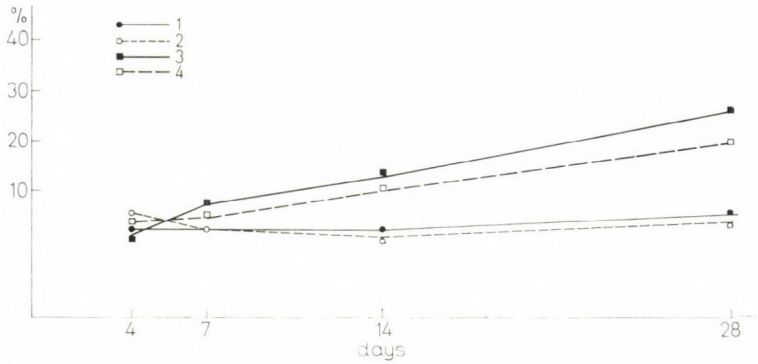


Fig. 7. Growth of the lettuce seedlings in the water extract of sandy cultures. Legend: the same as for Fig. 1

In both variants bentonite had the most significant influence on spore-forming bacteria (Fig. 3). Bentonite's stimulating effect must be the result of its buffering properties. As it is evident from Fig. 4, the tendency of pH corresponds to the tendency of the change in the number of bacteria. The pH degree of the aerated variants was only slightly higher and in the non-aerated variant almost one degree higher than in the control sample without bentonite.

Aerobic microorganisms grew, in the first place, in the aerated variants which, as is generally known, is the result of the decreased oxidative

Fig. 8. Number of bacteria developed on Thornton's media; soil variants. Legend: the same as for Fig. 1

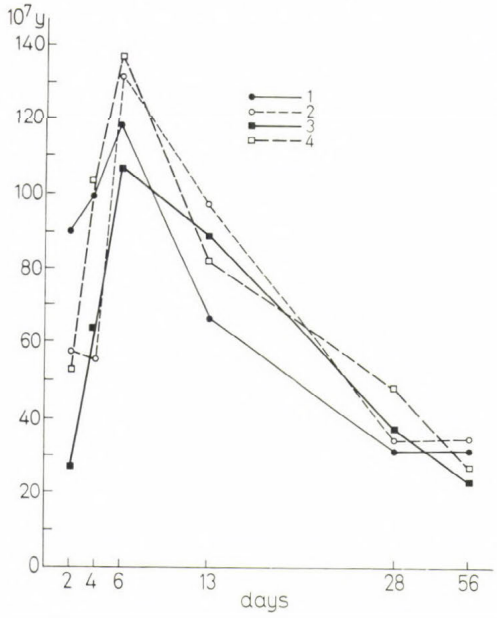
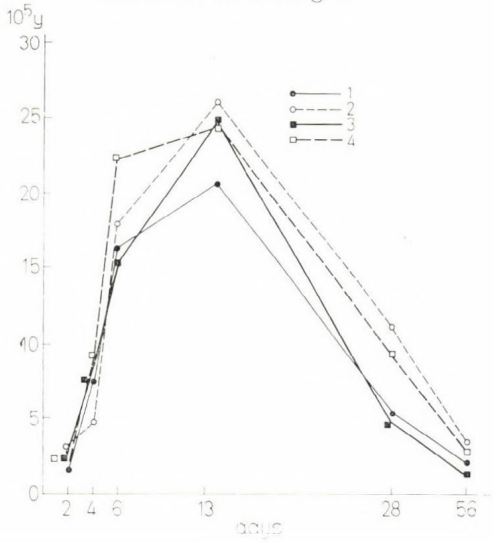


Fig. 9. Number of bacteria developed on meat-peptone agar; soil variants. Legend: the same as for Fig. 1



reduction potential of the non-aerated environment. *Actinomycetes* (Fig. 5) were discovered for the first time on the 7th day of the experiment in the aerated variants only. They were evidently stimulated by bentonite. Fungi (Fig. 6) developed more rapidly in the aerated variants. Their quantity in the non-aerated variants was stimulated by bentonite.

Water extracts of the studied substrates inhibited the growth of the roots of lettuce. It is evident from Fig. 7 that the extract from the non-incubated mixture caused 87% inhibition. The metabolites of microorganisms in the initial stage of decomposition of lucerne reduced in the further course of the experiment the growth of the lettuce roots to the lowest level for the whole period of experimentation. Gradually, but slowly, the reduction of inhibition was taking place; bentonite was responsible for the retardation of the process, showing evidently its sorption properties. In the non-aerated variants the inhibition did not show up considerably. At the beginning of the process of decomposition a slight stimulation by bentonite took place at the time when the bentonite was still able to adsorb the water-soluble metabolites.

Experiments with soil samples show that environmental conditions are much more complicated. Mineral and organic colloids and other components of the soil make its properties more constant. Initial concentration of its microorganisms is also higher than that of the sand and this is why it is

Fig. 10. Number of spore-forming bacteria on meat-peptone agar; soil variants. Legend: the same as for Fig. 1

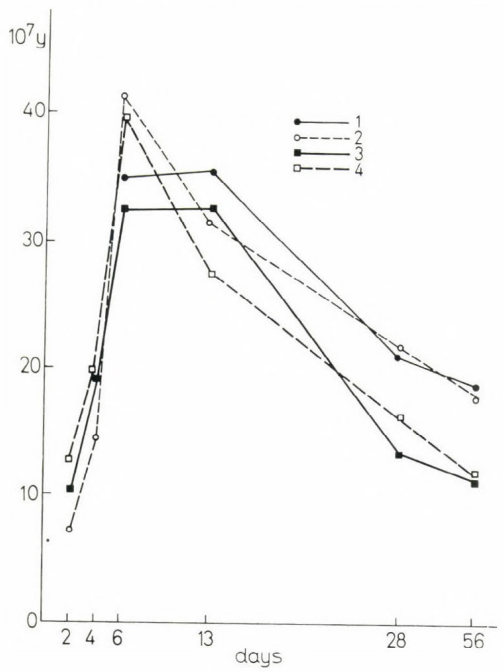
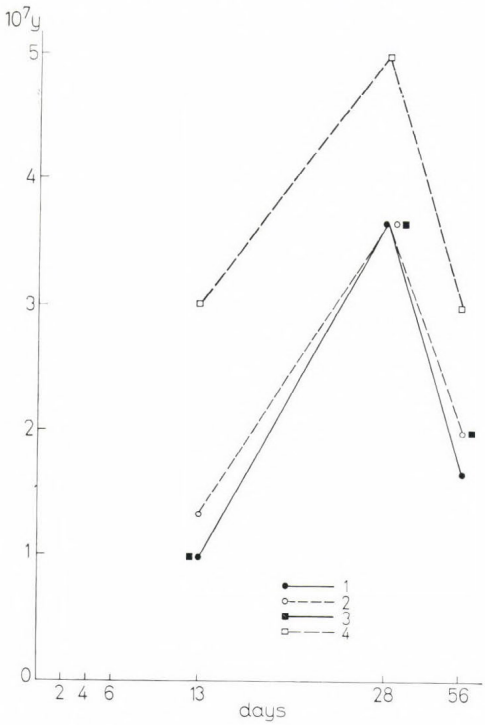


Fig. 11. Number of actinomycetes; soil variants. Legend: the same as for Fig. 1



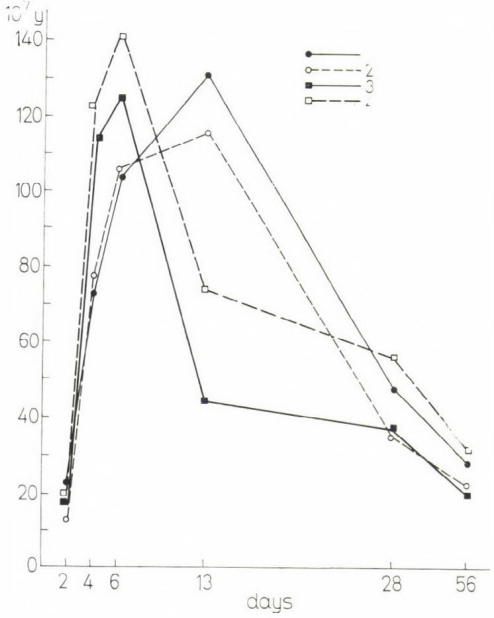


Fig. 12. Number of fungi; soil variants. Legend: the same as in Fig. 1

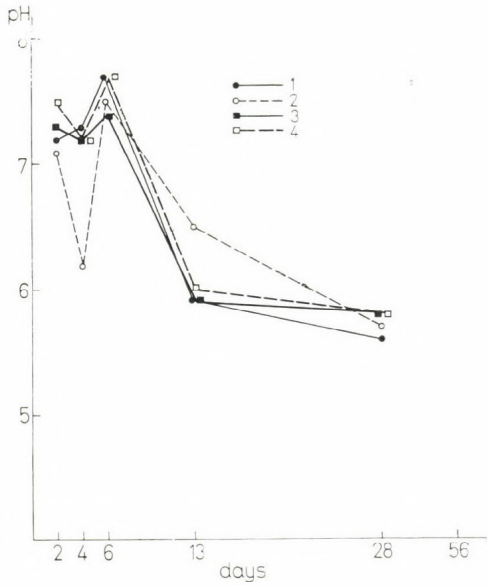


Fig. 13. Change of pH: soil variants. Legend: same as for Fig. 1

possible to expect faster decomposition of lucerne. This is shown in Fig. 8. The bacteria developing in Thornton's medium began to multiply very quickly and they reached their maximum on the 6th day of incubation (Fig. 9). It is possible to suppose that the soil itself is able to balance the insufficient supply of oxygen in the substrate in the initial stage. Aeration did not show such a remarkable effect as in the sand variant. These bacteria were stimulated by bentonite. The bacteria short of organic nitrogenous compounds developed faster in the aerated variants. In the non-aerated variants their maximum development was delayed by a week. Bentonite's stimulating inflence was observed only in the aerated sample.

Figure 10 shows the stimulating effect of bentonite on spore-forming bacteria only in the course of the first week of incubation. There were more spores in the non-aerated variant.

Actinomycetes (Fig. 11) began to multiply in the second week which corresponded to their development in the sand sample. They reached their maximum only after one month of incubation. Bentonite remarkably stimulated their quantitative growth. *Actinomycetes* multiplied in both variants of soil samples.

The development of fungi (Fig. 12) started from the beginning of incubation. They reached their maximum by the end of the 2nd week. They were stimulated by bentonite throughout the whole period. Their maximum development took place only after the maximum of bacteria had been reduced.

The dynamics of the pH in the soil variant was different from that in the sand. The maximum was observed on the 6th day (Fig. 13). From this time bentonite slightly increased the pH in both variants.

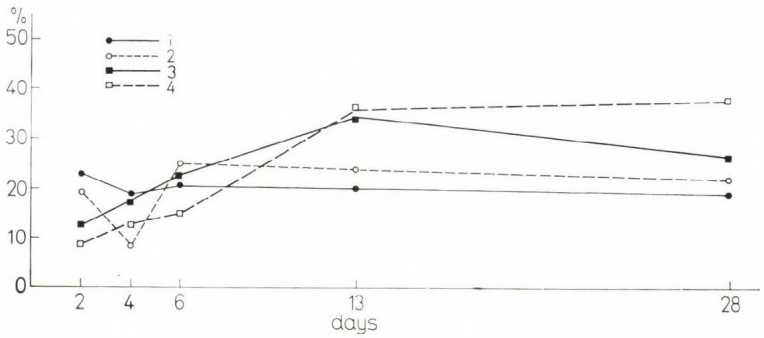


Fig. 14. Growth of lettuce seedlings in the water extract of soil cultures. Legend: the same as for Fig. 1

The growth of the plants (Fig. 14) was markedly inhibited but the inhibition was not extremely heavy. The aeration of the substrate and the addition of bentonite reduced the inhibition of the growth of the roots of lettuce. Nevertheless, the inhibition was larger in the first week of the experiment, but it was considerably lowered in the following period by bentonite — in the non-aerated variant by 78% and in the aerated sample by 62%. The soil extracts obtained from substrates with the highest number of bacteria showed the strongest effect. It must have been the water-soluble components of lucerne and the products of the metabolism of microorganisms that showed the effect since all the extracts were sterilized. Many researchers confirm the existence and formation of specific and non-specific substance which inhibit the plants' growth. This may be due to the products of metabolism of glycides and other substances, of organic acids (Takiyama 1964, Vang 1967, etc.), products of the hydrolysis of proteins, amino acids, peptides (Kubista 1965), saponins, aromatic and other substances (Mishustin 1955, Schoenbeck 1968, and others).

SUMMARY

Clay minerals together with organic matter of the soil influence considerably the physical, chemical and biological properties of the soil as well as its biochemical processes.

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ANTIBIOTICS AS ECOLOGICAL FACTORS IN THE DEGRADATION OF SOIL ORGANIC MATTER

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The financial results of field crop production depend, at least to a certain extent, on the properties and behaviour of the microbes living on, or in the neighbourhood of, plant roots. They may promote growth of the plants, but may retard or prevent it as well.

If the soil is in a good condition and microbial activity is high, organic residue will decompose rapidly providing the necessary conditions for humus formation. The concomitant increase in foodstuff supply and the production of germination or growth-promoting substance improve the situation for the plants and the increase in yield is then well established.

The adverse effect of the root rotting bacteria and other parasitic microorganisms can be explained in a similar way. The various toxic substances hinder the metabolism in proceeding on its normal course and the lytic enzymes destroy the tissue structures.

This kind of explanation seems rather convincing but it is oversimplified. It is actually much more complicated in nature.

Many types of microorganisms would live in the vicinity of plant roots but the plants exert a selective effect upon them. Root excreta favour one type of the whole population and supress the others. This means that the composition of the microflora varies from time to time around the plants according to the physiological state of the latter.

On the other hand, the microbes compete with each other for the foodstuffs excreted by the roots or supplied by other organisms.

In other words: a great deal of the mutual actions of plants and microbes can be created with well-known terms of ecology: symbiosis and antagonism. The phenomena which we can observe, however, represent only the composite results of many, mainly unknown processes.

I shall deal with only one type of the important biological processes, namely, antibiotic action.

Since the presence of antibiotics, as biologically active materials may primarily influence the development of the crop, their effect on the plants and on soil fertility has been thoroughly investigated. The results have been rather contradictory.

Many scientists take it for granted, that antibiotics are regularly found in the soils and they have a pronounced effect on the plants. According to them the same holds true for the degradation of organic residue.

On the other hand; there is a group of research workers who are doubtful of the importance of antibiotics. They suggest that antibiotics are easily

inactivated and therefore are not able to influence markedly either the organisms residing in the soil or their biochemical activities.

These contradictions are well established already in the earliest reviews in this field by Stallings (1954), Brian (1957) and Pramer (1958). The problem has been recently discussed by Beck (1968).

During the past years I investigated many Hungarian peat soils for the presence of antibiotics. I found only in a surprisingly few cases a true antibiotic effect. Even those strains which produce quite a lot of active substances under normal laboratory conditions failed to do so under natural circumstances. On account of this and similar observations I came to the conclusion that antibiotics do not always play such an important role as it is generally accepted. This paper presents a brief theoretical account supporting this view.

When considering some very simple cases which generally occur under normal soil conditions one might analyse the kinetics of the productions of antibiotics and its effects on the environment.

Let us observe two bacterial cells. One of them should produce a certain antibiotic and the other happens to be sensitive against this substance. For the sake of simplicity let us suppose that the generation time is equal for both cells and the production of active substance takes place only in the stationary phase, as generally the case is.

What will happen when these cells grow on the same soil particle or in the same hole? We may follow the events when plotting the number of the sensitive cells and the number of antibiotic molecules, respectively, against time.

Figure 1 shows the situation when the original cells begin to multiply simultaneously. The growth of the bacteria proceeds according to the usual exponential curve. Production of the antibiotic begins only when the log-phase is over and continues at a constant rate through a constant amount of cells. The increase in the number of the molecules will be represented by a straight line. The two lines never meet, that is, at any time the number of bacterial cells is greater than the number of antibiotic molecules.

Fig. 1. Increase of the number of sensitive cells and antibiotic molecules. N = number of cells

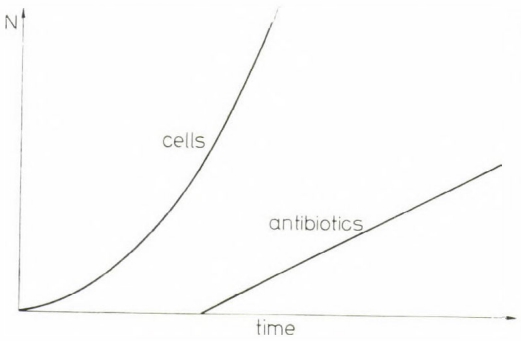
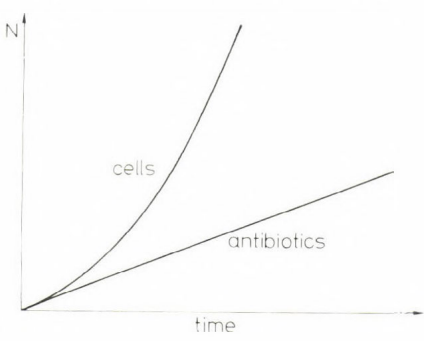


Fig. 2. The relationships when the producing cell begins to grow earlier but the production and growth of the sensitive cell begin at the same time. N = number of cells



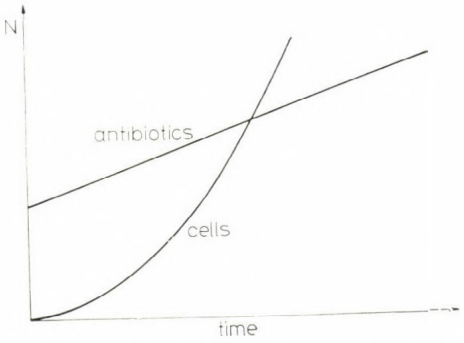


Fig. 3. Antibiotic production begins earlier than the growth of sensitive strain. N = number of cells

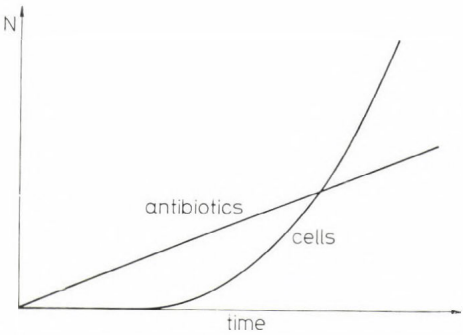


Fig. 4. A variation of the results demonstrated in Fig. 3. N = number of cells

It must be remembered, however, that for a true biological effect it is necessary that at least one molecule of the active substance must reach the surface of each bacterial cell. Without this close contact the bacteria would grow undisturbed and a colony would develop. There is no observable antibiotic effect even in the presence of the antibiotic.

Figure 2 shows the relationships when the producing cell begins to grow earlier but the production and growth of the sensitive cell begin at the same time. The number of the cells will always be greater and there is no antibiotic activity again.

Another case is to be seen in Fig. 3. Production begins earlier than the growth of the sensitive strain. Growth happens under the influence of a certain antibiotic. When this concentration exceeds the bacteriostatic value (about some hundred molecules per cell) growth will be prevented and a true antibiosis may be observed.

The case in Fig. 4 is only a variation of the aforementioned one. Where the curves cross over and up to this point there is the possibility of an antibiotic effect.

These considerations hold true and with slight modification can be applied to actinomycetes and the myceliar moulds.

Therefore, the conclusion may be drawn that bacterial growth is possible even in the presence of antibiotics, unless the antibiotic production begins much earlier.

In the case of any inactivation the number of the effective antibiotic molecules decreases further and the possibility of a true antibiotic effect will be even more restricted.

SUMMARY

According to my opinion the production of antibiotics by certain microorganisms does not influence the degradation of organic matter in soil so often as it is generally believed. I suggest rather that the amount of avail-

able foodstuff for the microbes has a more profound effect, and a better understanding of the chemical processes in the soil may be attained by considering the bioenergetic relationships.

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EFFECT OF ORGANIC AND INORGANIC FERTILIZERS ON SOME MICROBIOLOGICAL AND BIOCHEMICAL PROPERTIES OF THE SOIL

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The addition of NPK nutrients to the compost take part in the transformation of the organic matter. It may be assumed that, after their working in, microbiological and biochemical changes will take place in the soil differing from those occurring in the case of a separate working in of organic fertilizers and NPK nutrients.

MATERIAL AND METHODS

In a field vegetation experiment the influence of two composts (Table 1) on the yield of potatoes was investigated and simultaneously, certain microbiological and biochemical changes in the soil were evaluated. The

Table 1

Technological, chemical and biochemical characteristics of composts applied in the experiments

		Compost A	Compost B
Raw materials			
Town refuse	in %	60	60
Moor	in %	13	13
Lignite	in %	13	13
Sludge wastes	in %	14	12
NPK fertilizers	in % N	—	0.467
	P_2O_5	—	0.487
	K_2O	—	1.126
Total organic substances in %*		42.19	40.68
Total N in %*		1.10	1.25
P_2O_5 in % acc. to Égner*		0.60	1.35
K_2O in % acc. to Schachtschabel*		0.70	1.79
B (mg CO_2/100 g of dry matter/hour)		3.46	2.85
NG:B (relative numbers)		6.30	15.50

* = All data are referred to dry matter.

variants of the experiment were as follows: 1) control (a control plot without any fertilization), 2) a plot fertilized with NPK only, and plots fertilized, 3) with 30 metric tons of compost A, 4) with 30 metric tons of compost

A and NPK fertilizers per hectare, 5) with 30 metric tons per hectare of compost B, and 6) with 90 metric tons of compost B per hectare. The NPK dose had been calculated for supply fertilization and consisted of 140 kg of N, 146 kg of P_2O_5 and 336 kg K_2O. This dose of nutrients was also added to the 30 metric tons of compost B at the beginning of its maturing.

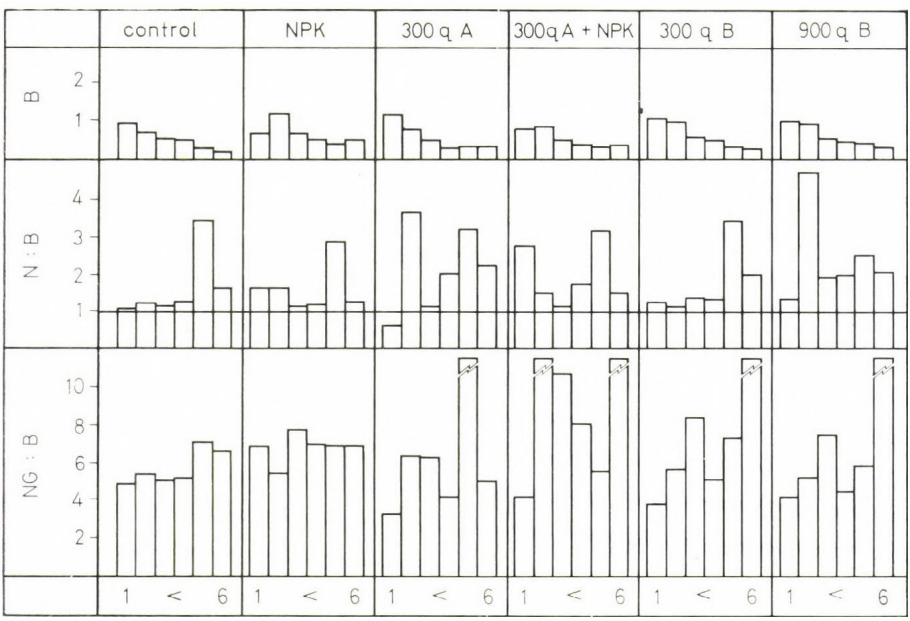
In the course of the vegetation growth soil samples were taken from the experimental parcels, in which, after sieving through a 2 mm sieve, certain cultivation groups of microorganisms were examined (Kozová, Novaková 1956), respiration rate (Novák, Apfelthaler 1964), and nitrogen content (Pokorná-Kozová et al. 1964) were determined.

RESULTS AND DISCUSSION

In the course of the whole investigation the basal respiration of the soil samples taken from the different experimental parcels changes only insignificantly. This means that even a dose of 90 metric tons of compost B per hectare did not markedly change the production of CO_2 (Fig. 1).

However, the relative respiration N:B (Fig. 1) indicates substantial changes of the potential N respiration due to the addition of different organic fertilizers. The increase of relative respiration up to even 1.0 after fertilization with both composts A and B indicates a lack of physiologically utilizable nitrogen, which, in the case of a dose of 30 metric tons per hectar is higher in compost A than in compost B. The NPK fertilizers supplied

Fig. 1. Respiration of the respective variants of the experiment of 6 sampling series taken during the vegetation season (B in mg CO_2/100 g dry matter/hour; relative respiration N:B, NG:B in relative numbers)



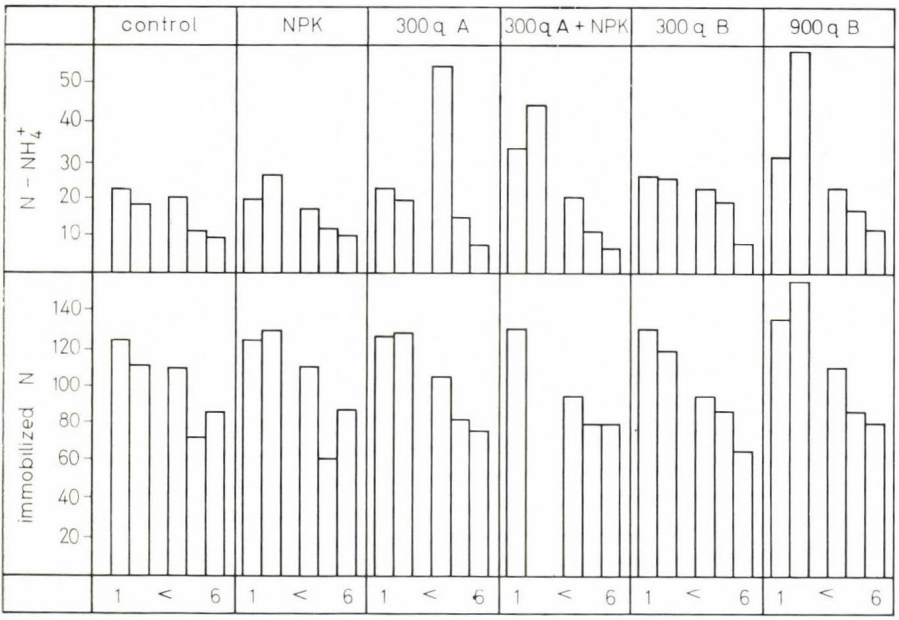


Fig. 2. Content of NH_4^+—N and immobilized N in 6 series of soil samples taken during the vegetation season (in mg %)

to compost A lowered the relative N:B respiration, but not to the level of compost B with surplus fertilization. After a dose of 90 metric tons of compost B per hectare the relative respiration N:B increases substantially compared to that of 30 metric tons per hectare and it is obvious that the lack of physiologically available nitrogen did not change during the whole course of investigation.

After working both composts A and B, the NH_4^+—N content (Fig. 2) was higher than in the control soil and with only NPK fertilization. Industrial NPK fertilizers added to compost A substantially increased the NH_4^+—N content, which was shown in the already mentioned decreasing of the relative N:B respiration. A dose of 30 metric tons of compost B with supply fertilization increased the NH_4^+—N content compared with the sole compost A, but not to the level of compost A with NPK fertilizers. A dose of 90 metric tons of compost B increased the NH_4^+—N content to the level of compost A with NPK fertilizers. From the results obtained it can be seen that the NH_4^+—N content does not always correspond to the relative N:B respiration and that particularly in the case of a dose of 90 metric tons of compost B. This difference may be explained as having been caused by the immobilization of NH_4^+—N into organic compounds, as in the case of a dose of 90 metric tons of compost B the quantity of bonded NH_4^+—N increases markedly (Fig. 2).

The relative N:B respiration and also the changes of the NH_4^+—N content in some of the soil samples show that in the soil there occurs a decomposition of organic substances contained in the different composts.

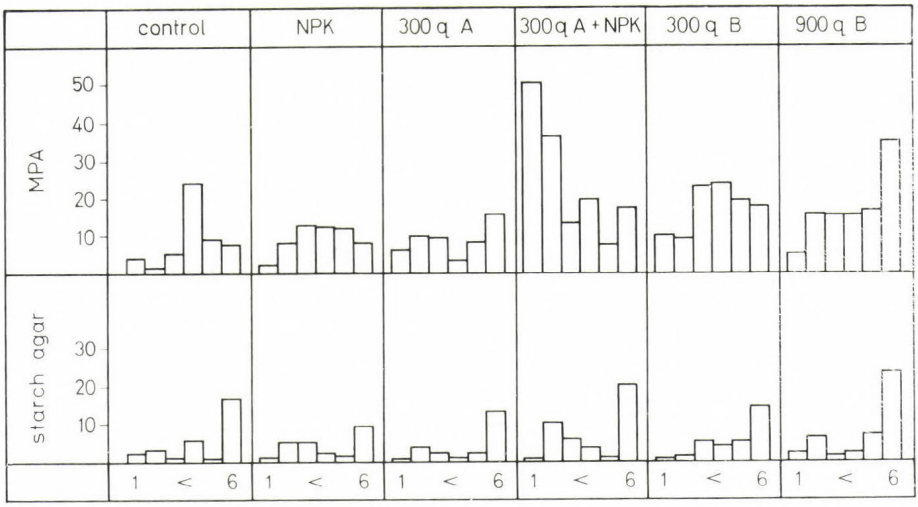
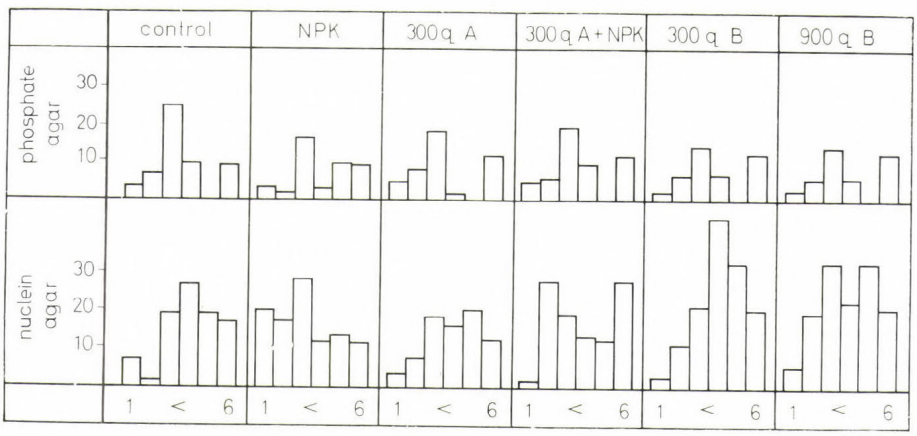


Fig. 3. Number of microorganisms in 6 series of soil samples taken during the vegetation season (in mill./1 g dry matter)

This is indicated also by the stability of organic substances characterized by the relative NG:B respiration, which is the highest after fertilization with compost A+NPK fertilizers, and the lowest after a dose of 90 metric tons of compost B. Relative respiration increases after fertilizers together with the prolongation of the vegetation period more regularly after compost B than after compost A and compared with the almost unchanging relative respiration of the non-fertilized control and with the control fertilized only with NPK fertilizers.

With the changes of respiration and with the change of the NH_4^+-N content are correlated also the determined quantities of microorganisms

Fig. 4. Number of microorganisms in 6 series of soil samples taken during the vegetation season (in mill./1 g dry matter)



of the investigated cultivation groups. These are particularly bacteria growing on MPA (Fig. 3), whose quantity increases after fertilization with compost A + NPK fertilizers and also after both doses of compost B. A difference in the number of these bacteria after fertilization with composts A and B is shown also by the fact that after application of compost A their quantity is largest at the beginning of vegetation growth and decreases gradually; whereas after application of compost B, on the other hand, it increases with the time of vegetation. The applied organic fertilizers influenced the number of bacteria growing on a starch agar to a much lesser extent. It is, however, possible to observe a substantial difference in the representation of bacteria growing on a phosphate and nuclein agar (Fig. 4). After organic fertilization the quantity of bacteria on the phosphate agar decreases slightly if compared with the bacteria growing on the nuclein agar. The number of bacteria on nuclein agar of both composts shows the same trend as does the number of bacteria on MPA.

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THE INFLUENCE OF DEEP PLACING OF ORGANIC MATERIAL ON SOME GROUPS OF SOIL MICROORGANISMS

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The experiments with deep placing of farmyard manure in sandy soils have been carried on in Poland since 1956. Farmyard manure applied at a rate of 600 q/ha was placed 60 cm deep; there it forms a layer about 1 cm thick and brings about considerable changes in the soil profile. First of all, the water relations change, the total quantity of roots increases and their range extends into deeper layers. Owing to the inset of farmyard manure the processes of organic matter decomposition follow not the direction of oxiditation and mineralization but humification, and the mineral components get trapped in the arable layer and are not washed out into deeper layers.

The objective of microbiological researches was to find out how the application of deep drainage, and particularly the addition of a large quantity of farmyard manure, influenced the changes in the number and activity of some physiological groups of microorganisms and their distribution in the soil profile.

MATERIAL AND METHODS

Investigations on the experiment with deep placing of farmyard manure were carried out in two series.

Series I was made in the first, second and third year after the experiment had been laid out. Samples for microbiological analyses were taken from the following treatments:

a) 60 cm deep ploughing + 600 q/ha farmyard manure + Ca
b) 50 cm deep ploughing — without farmyard manure + Ca
c) 20 cm deep ploughing + 300 q/ha farmyard manure
d) 20 cm deep ploughing — without farmyard manure + Ca
e) 60 cm deep ploughing + 600 q/ha farmyard manure

The samples were taken from two horizons (15—20 cm and 50—55 cm) every two weeks during the vegetation period. Samples from the horizon 15—20 cm were taken from 15 spots in the plot by means of a stick soil sampler. The samples were mixed and then an average was taken. Samples from the horizon 50—55 cm were taken from 6 spots in the plot by means of a soil borer, these having been also mixed, an average was taken. Microbiological analyses were carried out on the next day.

Microbiological determinations were made by the method of Pochon consisting in determining the activity of several physiological groups. For this purpose the series of liquid media of composition adjusted to the needs

of several physiological groups were inoculated with soil suspensions of various dilutions. The incubation was run for 14 days at 27 °C. Within that period there was determined the loss of substance being the medium for a given group as well as the products of metabolism of the microorganisms. The results helped to plot curves reflecting the intensity of bacterial action. The test comprised two groups of microorganisms:

A) bringing about nitrogen transformation
1. decomposing protein
2. ammonifiers
3. fixing free nitrogen

B) bringing about carbon transformation
1. decomposing starch
2. decomposing pectins
3. decomposing cellulose

The amount of *Azotobacter* and cellulose-decomposing bacteria were determined by Winogradsky's method. 25 additional analyses were made to determine the amount of fungi and actinomyces.

During three vegetation seasons there were made as many as 80 analyses of samples taken from 20 cm depth, and 66 analyses of samples taken from 60 cm depth. Diagrams 1—9 representatively exemplify the results obtained.

Series II was made ten years after the experiment had been laid out, to check how long the application of deep drainage acted on the changes in distribution and activity of some groups of soil microflora in the profile. Determinations were made in following treatments:

a) 20 cm deep ploughing — without farmyard manure + Ca
b) 20 cm deep ploughing + 300 q/ha farmyard manure + Ca
c) 60 cm deep ploughing + 600 q/ha farmyard manure + Ca

The samples were taken from the horizons 10—20 cm, 20—40 cm, 40—60 cm and 60—80 cm. Six samples taken from each horizons were mixed together and then in the average sample were determined:

a) the number of bacteria decomposing cellulose by the Winogradsky's "a graine" method and by the dilution method,
b) the number of *Azotobacter,*
c) distribution of microorganisms in the soil profile by Mishustin's method.

RESULTS

When comparing the results it was observed that those obtained in 1959 considerably differed from those obtained in the years 1960 and 1961. The results of the latter two years are quite the same.

Decomposition of protein. Decomposition of gelatin, which was used as a test of protein decomposition, was most intensive in treatment 1, and less intensive in treatments 5, 2, 3 and 4. These results kept throughout the vegetation period, with slight deviations in the samples from both depths (Fig. 1).

In the years 1960 and 1961 the results in all treatments and from all depths were nearly the same with a slight tendency to increase in treatment 1.

Ammonification. The decomposition of alanine in all the examined samples was rather rapid. It was the most intensive in treatment 1, then in

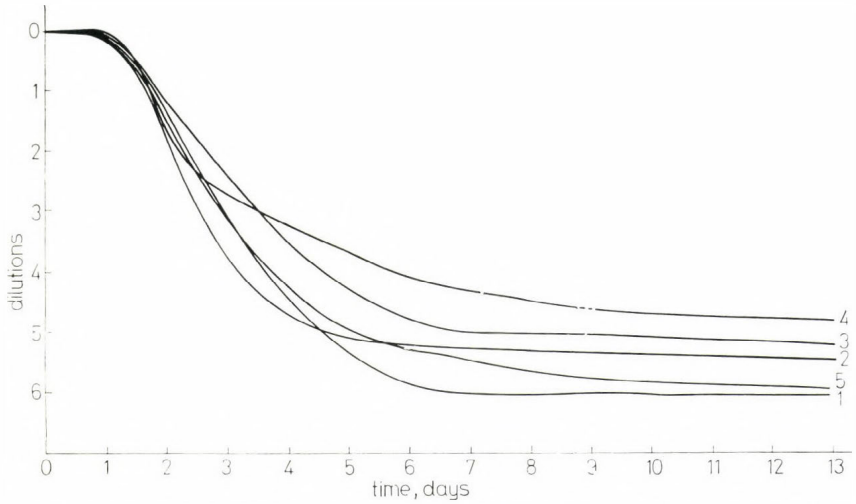


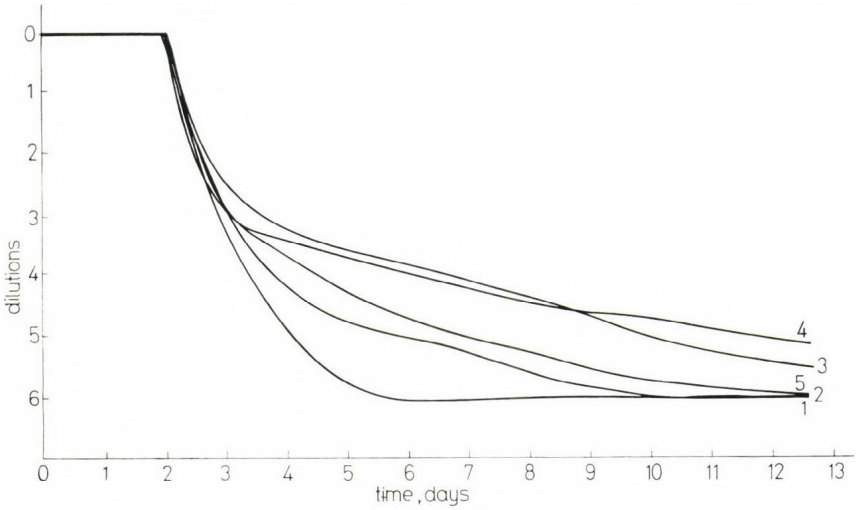
Fig. 1. Decomposition of gelatin. 1) 60 cm deep ploughing + 600 q/ha farmyard manure + Ca; 2) 50 cm deep ploughing without farmyard manure + Ca; 3) 20 cm deep ploughing + 300 q/ha farmyard manure + Ca; 4) 20 cm deep ploughing without farmyard manure + Ca; 5) 60 cm deep ploughing + 600 q/ha farmyard manure

treatment 2, the results of the remaining treatments being nearly the same. Similar values were obtained in the samples from the 60 cm horizon (Fig. 2).

In the years 1960 and 1961 no important differences between the treatments were observed.

Denitrification. In the group of bacteria denitrifying nitrates no differences were observed between the treatments in the years 1959, 1960 and 1961 (Fig. 3).

Fig. 2. Decomposition of alanine. 1)—5) = the same as in Fig. 1



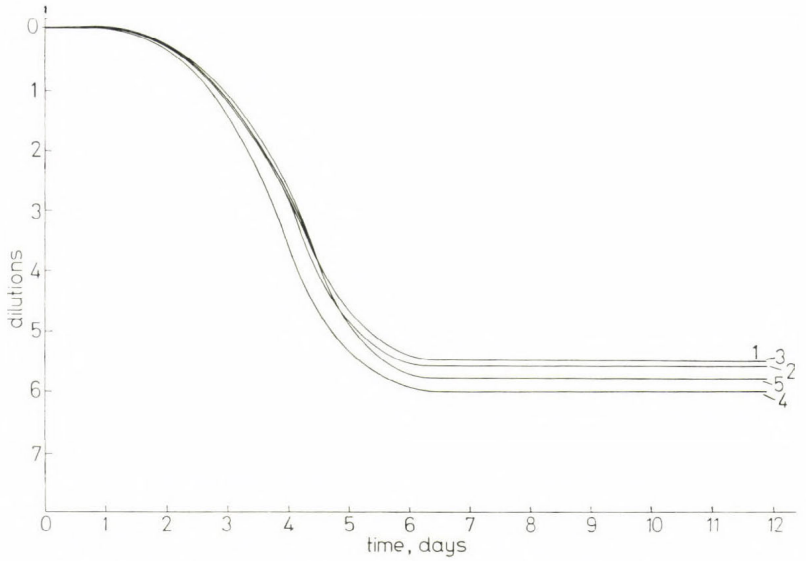


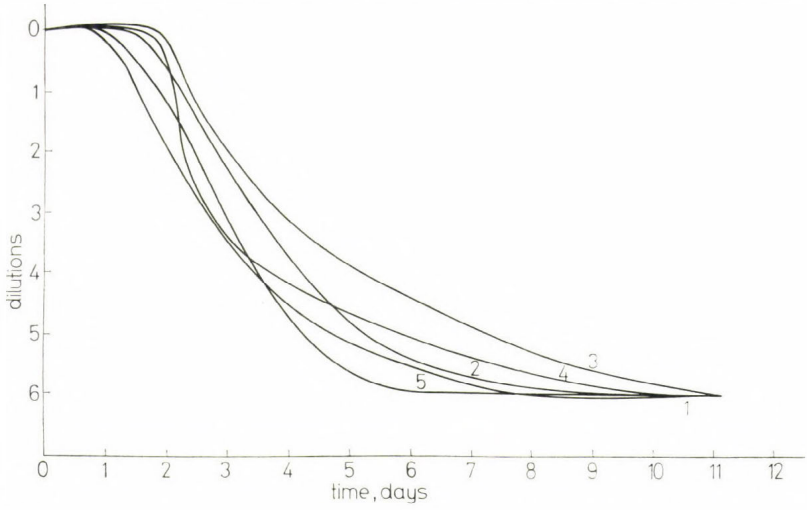
Fig. 3. Denitrification. 1)—5) = the same as in Fig. 1

Free-living N-fixing bacteria. Azotobacter appeared only twice in treatment 1.

Carbon transformation. Decomposition of starch. The decomposition of starch was rather rapid in all treatments. It was the most intensive in treatments 1 and 5, the results in the remaining treatments having been the same. Fig. 4 is representative for the depths 20 and 60 cm.

In the years 1960 and 1961 the differences between treatments were slight.

Fig. 4. Decomposition of starch. 1)—5) = the same as in Fig. 1



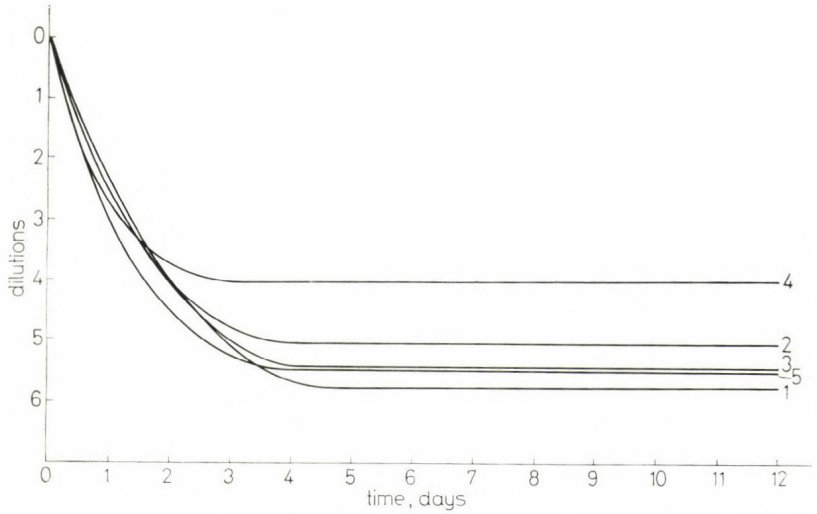
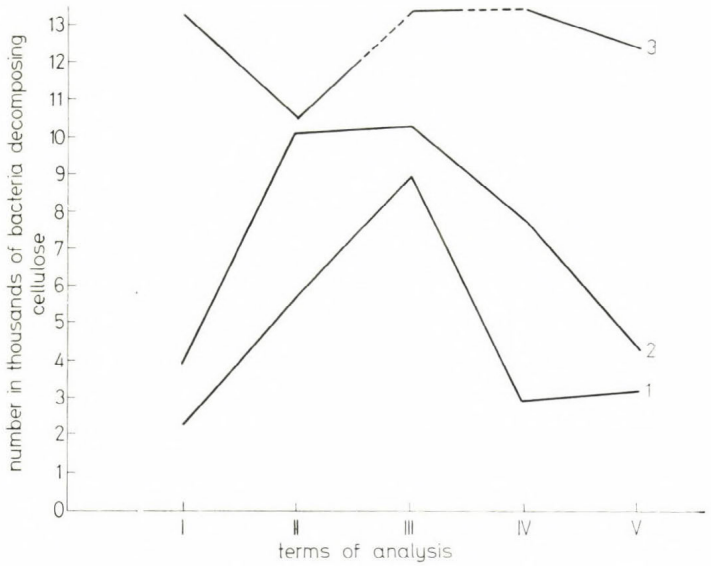


Fig. 5. Decomposition of pectins. 1)—5) = the same as in Fig. 1

Decomposition of pectins. The strongest decomposition of pectins was observed in treatment 1, and then in treatment 5, 3 and 2, and the weakest in treatment 4 (Fig. 5).

Fig. 6. The number of cellulose-decomposing bacteria. Sampling depth 0—20 cm. 1) 20 cm deep ploughing without farmyard manure + Ca; 2) 20 cm deep ploughing + 300 q/ha farmyard manure + Ca; 3) 60 cm deep ploughing + 600 q/ha farmyard manure + Ca



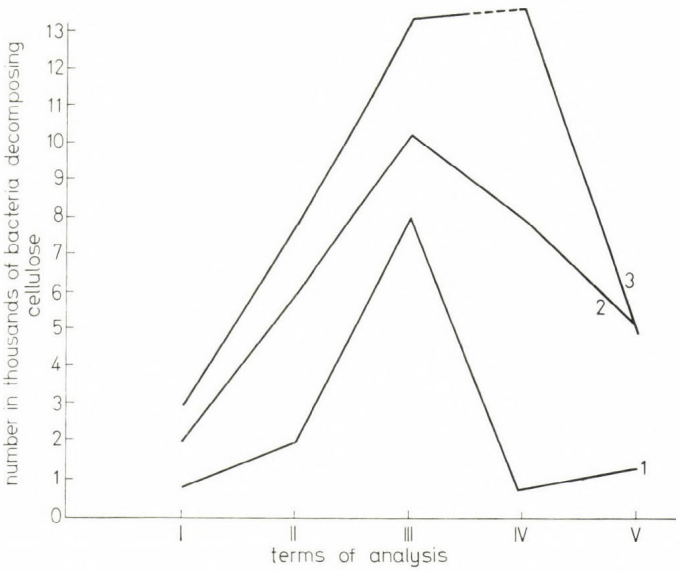
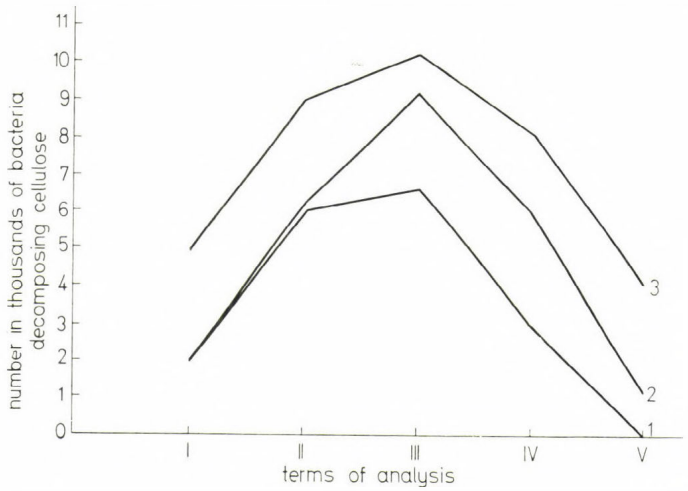


Fig. 7. The number of cellulose-decomposing bacteria. Sampling depth 20—40 cm. 1)—3) = the same as in Fig. 6

Decomposition of cellulose. The results were uneven; there occurred a considerable quantity of fungi decomposing cellulose, due to high acidity of the soil.

Bacteria decomposing cellulose. The number of bacteria calculated by two methods was nearly the same, so only those obtained by the first method are discussed and shown in Figs 6—9. The number of cellulose decom-

Fig. 8. The number of cellulose-decomposing bacteria. Sampling depth 40—60 cm. 1)—3) = the same as in Fig. 6



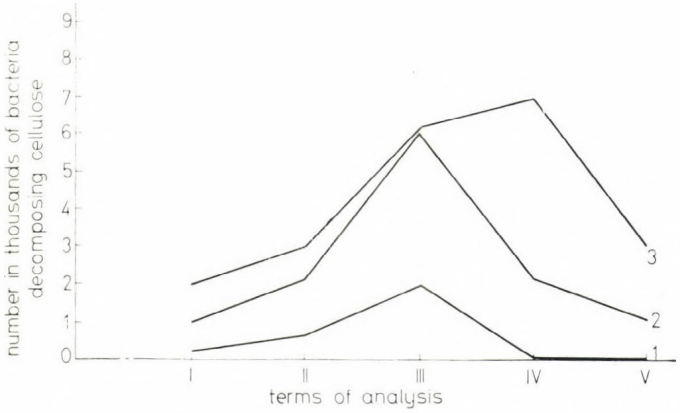


Fig. 9. The number of cellulose-decomposing bacteria. Sampling depth 60—80 cm. 1)—3) = the same as in Fig. 6

posing bacteria in the examined treatments have been shown in diagrams by horizons. In all the horizons the lowest quantity was observed in treatment 1 (without farmyard manure), and the highest one in treatment 3 (600 q/ha farmyard manure ploughed in deeply); the quantities in treatment 2 (300 q/ha farmyard manure ploughed in superficially) were intermediate between treatments 1 and 3. The influence of farmyard manure ploughed in deeply, is marked and brings about an increase of the quantity of bacteria decomposing cellulose by 10 to 30%, this being of great importance in light soils.

Azotobacter. *Azotobacter* was accepted as the index of soil fertility in order to check whether under the influence of deep treatment with farmyard manure the ecological conditions had changed so as to favour the development of *Azotobacter*. Fivefold analyses from all treatments and horizons did not reveal its presence. The absence of *Azotobacter* is due to still low pH and low fertility of the soil.

Distribution of microorganisms in the soil profile — Mishustin's method. This appeared to be a very good test of the distribution and activity of microorganisms in the soil profile up to 45 cm depth. The action of microorganisms as well as that of cellulose decomposing bacteria may be estimated on basis of the observations made on the stripes of canvas having been put into the soil.

Treatment 1. In this treatment an intensified action of microorganisms was observed only in the horizon 5—20 cm, while farther sections of the canvas, except some small areas, were not "attacked" by microorganisms at all. The surface of the canvas from 5 to 20 cm was damaged by the cellulose decomposing bacteria.

Treatment 2. In this treatment a positive effect of farmyard manure on the development of soil microorganisms was distinctly marked. In the horizon 5—20 cm as well as in the deeper ones (20—40 cm) considerable losses of canvas were observed as well as numerous yellow and black spots testifying the presence of cellulose decomposing bacteria and fungi.

Treatment 3. The development of microorganisms in this treatment was the strongest compared with the preceding ones. Farmyard manure applied as an inset pronouncedly activated and multiplied the soil organisms. The losses of canvas are visible all over its surface, and there are a lot of yellow, orange and black spots pointing to an intensive work of cellulose decomposing bacteria and fungi.

CONCLUSIONS

1. In the first year after the experiment had been laid out, the addition of 600 q/ha farmyard manure and deep ploughing brought about pronounced increase of the activity of soil microflora in all the physiological groups under examination.

2. In the next two years the influence of farmyard manure was somewhat less, but a tendency to the activation of microorganisms was still observed.

3. The influence of deep placing of farmyard manure ten years after it had been ploughed in would point to its positive and lasting effect on some groups of microorganisms. 600 q/ha of spot placed farmyard manure intensified the action of microorganisms throughout the soil profile and secured more favourable conditions for their development.

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SOIL MICROBIOLOGICAL ACTIVITIES AND THEIR RELATIONS TO OTHER SOIL-PARAMETERS

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Soil enzymes are biocatalysators primarily of microbiological origin, to a lesser extent also derived from plants. They regulate metabolism in the soil, especially the turnover of organic substances. Within these processes enzymes have for instance a great influence on release and accumulation of nutrient substances and they regulate gas exchange. They are very important to the synthesis of new organic materials or groups of materials, perhaps of humic substances, and so they participate to a high degree in the formation of organo-mineral compounds and chelates in soil, and in this way they have a great influence on soil structure. Accounted with that are soil enzymes together regulating composition dynamics and functional effectiveness of microbiocoenoses in soil. (For detailed description see Rawald, in print.) It should be considered that soil enzymes with their activities play an essential role in the biological activity of soils. They are also very important from the point of view of soil fertility. This is why we studied the effects of organic and mineral fertilizing on the activities of some selected enzymes (polyphenoloxidase, tyrosinase, catalase, dehydrogenases) and compared the effects on several soil microbiological (density of bacterial population) and soil chemical parameters (total carbon content quotient Q 4/6).

In the following 5 permanent fertilizing experiments (PFE) we have investigated in each case the deficient variant (without any fertilizing = a), the complete fertilizing variant (farmyard manure with NPK = b) and the mineral fertilizing variant (only NPK = k):

heavy soils:	CL	(Lößkert-Parabraunerde)
	MI	(Ton-Ranker)
black earth:	LA	(Löß-Schwarzerde)
light soils:	MÜ	(Tieflehm-Fahlerde)
	GK	(Bändersand-Braunerde)

Polyphenoloxidase was determined with a modified method according to Kozlov (1964), dehydrogenase modified according to Lenhard (1957) (see in Böhme, Rawald and Stohr 1969, Rawald 1968a, 1968b). Tyrosinase was determined according to Luthardt and Lyr (1965), catalase modified according to Ambroz (1956).

Bacteria population was determined by means of fluorescence microscope (Rawald 1968a, Stohr). Methodical information about determination of the total carbon content, of colorimetrical quotient Q 4/6 as well as of the rate of undecomposible carbon content are to be found in publications of Domke and Rawald (1969) and Rawald (1968a).

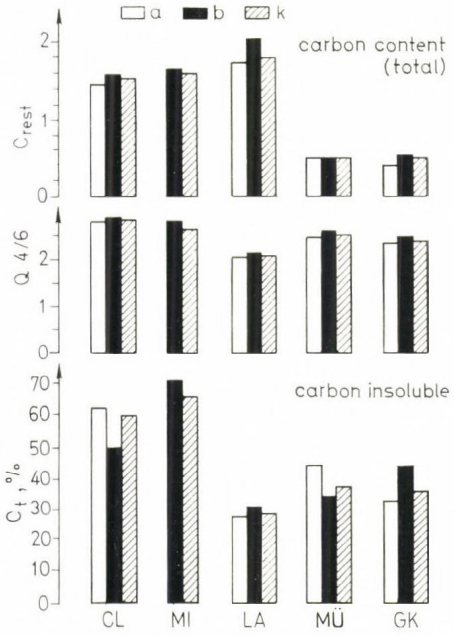
One part of the investigations has been carried out in their annual dynamics, i.e. on 5, terms, from March to November. Other researches were carried out with annually homogenized samples which were produced by intensive mixing of 5 equal samples from 5 terms. The results of investigations and the stability of occurrences allowed this rational method (Rawald 1969).

RESULTS AND DISCUSSION

In oder to mark the soil chemical composition in the experimental area, the effects of fertilizing on some soil chemical parameters of all the researched PFE and variants of fertilizing are represented in Fig. 1.

Concerning total carbon contents (C_t) both heavy soil habitats CL and MI as well as the black earth habitat LA have much higher values than both light soil habitats MÜ and GK. The content of carbon, insoluble in NaOH (C_{rest}), however, differs from these values; again, indeed, both heavy soil habitats GL and MI have the highest values, but both light soil habitats have proportionally much higher values, and even the black earth habitat has the lowest values concerning this insoluble carbon fraction. With regard to the colour quotient (Q 4/6) relatively the investigated permanent fertilized habitats do not much differ; the heavy soil habitats (CL, MI) have only slightly higher values than the light soil habitats (MÜ, GK), the black earth habitat has the lowest values again. It can be established that the results of researching total carbon content are in an inverse ratio to the results of researching Q 4/6 and the content of insolube carbon.

Fig. 1. Carbon (total) content, Q 4/6 values and carbon insoluble in soils of permanent fertilizing experiments. (According to Domke.) Legend:
a = without manuring; b = farmyard manure + NPK; k = only NPK



Fetilizing methods have effect on the investigated parameters in the following way: complete fertilizing (manure with NPK) as a rule induces higher C_t-contents than it is to be established in deficient variants (without any fertilizing); mineral fertilizing (NPK) induces C_t-values between theones of the variants with complete fertilizing and variants without fertilizing. Similar are the values of Q 4/6, even if the differences are smaler. All the habitat fertilizing methods do not have the same effect on the contents of insoluble carbon. Sometimes the total carbon contents are smaller in the variants of complete fertilizing than in the variants of mineral fertilizing or without fertilizing (CL, MÜ); in

other habitats the C_{rest}-values of the variants with complete fertilizing are higher than those of the variants with mineral fertilizing only (MI, LA, GK); these values for their part are in the complete fertilizing variant of several PEF higher than those of variants without fertilizing (LA, GK), at other habitats, however, they have lower values (CL, MÜ).

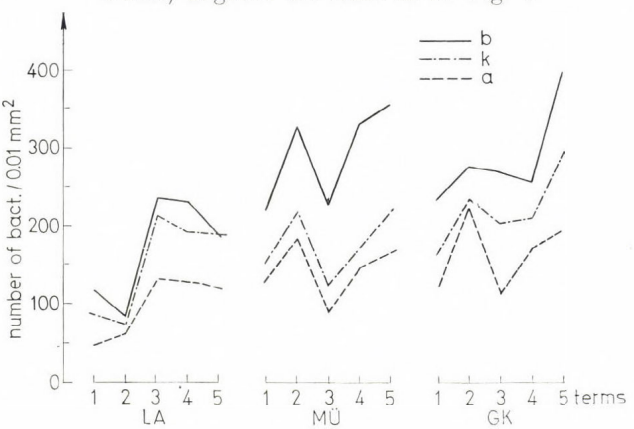
We suggest these soil chemical parameters be selected from many others, but they should not be interpreted as a functional meaning of soil enzymes for metabolism and especially not without comparison with other, such as biological and chemical parameters of the soil. It is impossible to test and to estimate the effect of cultivation methods in other ways in the case of organic and mineral fertilizing with only a single parameter or a group of similar parameters. Generally speaking a conclusion cannot be drawn from the value of one soil parameter to those of other parameters — unless there are direct, substantial interactions as there are in the case of cellulose and activity of cellulase (Rawald 1968b).

All biologically directed processes in the natural habitat are subject to changes with annual dynamics which can be very considerable. As an example the development of the density of bacterial population during the period of vegetation (from March to November) at 3 of the investigated permanent fertilized habitats (LA, MÜ, GK) is represented in Fig. 2. It can be seen that the value of bacterial population (number of bact./0.01 mm^2) of the variant of mineral fertilizing is to be found among the values of other variants during the whole year. The annual average level in black earth (LA) is considerably lower than that in light soil (MÜ, GK).

The soil enzymatic activities are subject to changes in the annual dynamics too. Figure 3 shows the dynamic annual development (from March to November) of polyphenoloxidase and dehydrogenases in 2 habitats. According to synthesis, biological decomposition, desactivation by chemical combination or sorption, reactivation and so on, it is not to be expected that there are direct relations between the dynamic yearly development of any functional or systematical group of microorganisms, on the one hand, and the yearly development of the activity of an enzyme or a group of enzymes on the other. It must be considered, however, that enzymes are substances of microbial origin, and even if direct relations are not expected there are common tendencies in the yearly development.

Figure 3 shows through the case of the bacterial population — that the annual average level of enzymes in black earth (LA) is

Fig. 2. Seasonal dynamics of the density of bacteria populations (referred to 1 g of organic carbon) in soils of permanent fertilizing experiments. (According to Stohr.) Legend: the same as in Fig. 1



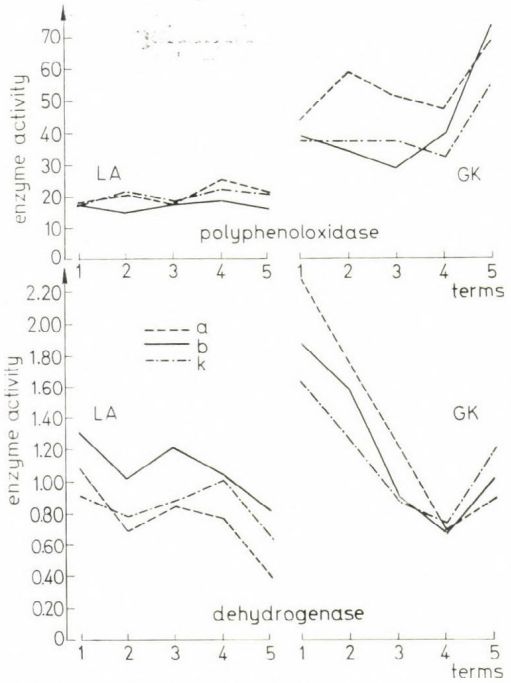


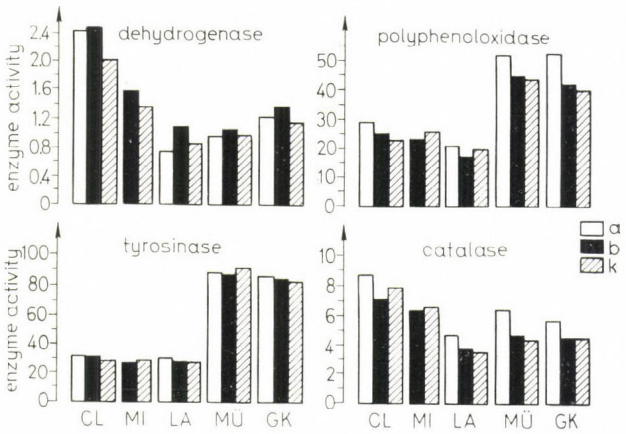
Fig. 3. Seasonal dynamics of two enzymes (referred to 1 g of organic carbon) in soils of two fertilizing experiments.) Legend: the same as in Fig. 1

lower than in light soils (GK). In black earth (LA) the annual dynamics of dehydrogenase is similar in the 3 variants, this is quite the same as in the case of the bacterial population; that means the curves are nearly of the same kind and the variant with complete fertilizing has higher values than the deficient variant, and the variant with mineral fertilizing ranges among the others. In case of dehydrogenase activity such a complete concurrence was not observed in light soil (GK).

Obviously it is necessary to consider polyphenoloxidase otherwise than the group of dehydrogenases, concerning not only its functional meaning but also its dependence on fertilizing methods. Organic fertilizing can influence synthesis or activity of polyphenoloxidases less intensively or possibly depressively, because the average values of the variant of complete fertilizing are lower than those of the variant without fertilizing, and the variant with mineral fertilizing is on the average level compared with the others.

The representative average values of annual mixed samples of fertilizing variants from the investigated permanent fertilizing experiments are summarized in Fig. 4. All the values are referred to total carbon content (C_t), that means to the humus content. Thereby it is possible to compare directly the habitats concerning humus quality; these comparisons are not

Fig. 4. Intensity of the activity (annual means of 5 terms a year) in soils of permanent fertilizing experiments. (The activity values are referred to 1 g of organic carbon)



bounded with relations of humus content. Referring to 1 g soil there are entirely different relations between the habitats.

In case of polyphenoloxidase and tyrosinase both habitats in light soils (MŰ, GK) clearly separate from the habitats in heavy soils (CL, MI) and from the black earth habitat with more than doubled values; in case of dehydrogenase and catalase the present values do not allow such a conclusion. Under these conditions the activity of microorganisms, the clay content, the sorption and the desactivation are all affected.

Concerning the influence of organic and mineral fertilizing on soil enzymatic activities, in case of dehydrogenase the variants with manure + NPK have the highest activity values in each case. The values of the deficient variants and the variants with NPK only are in different relation to each other before without any relation to the soil. In one case the higher values belong to the deficient variant, in the other cases to the NPK variant.

In case of tyrosinase the feature is extensively equalized, that means between the 3 variants of fertilizing there are only small differences.

In case of polyphenoloxidase and catalase, however, the highest activity values are to be found in each case in the deficient variant (without fertilizing); while the relations between the variants with complete fertilizing and with NPK-fertilizing are very different. The reason for this phenomenon could be found in the sphere of sorption conditions. In evaluating the fertility of soil, and in this special case estimating the effect of organic and mineral fertilizing of soil does not mean that an isolated supervision of bacterial population and other soil biological values (physiological or systematic groups, soil chemical parameters as well as this or that soil enzymatic activity) cannot have been successful. On each case the recording of functional values, such as special enzyme activities, intensity of respiration and intensity of nitrogen fixation, allow such establishments in the best way. But even each of these parameters would not be sufficient, because in each case only a small part, a sector of the highly complicated and complex processes of metabolism can be evaluated.

Organic and mineral fertilizing have a different influence on the parameters which are investigated within the bounds of these experiments. Moreover, in case of total carbon content (C_t), colour quotient (Q 4/6), bacterial population and dehydrogenase activity it can be shown that much higher values are obtained in variants with manure and NPK fertilizing than in variants with only mineral fertilizing. But in case of total carbon content, colour quotient and bacterial population an intermediate position of the values affected by mineral fertilization is established between the values effected in opposition to these by complete fertilizing (manure + NPK) and the deficient variant; in case of dehydrogenase activity changing proportions between deficient variant and mineral fertilizing variant are established.

In case of the other redoxase activities direct relations between fertilizing and single activities which could be generalized even for one enzyme, cannot be established.

How complexly dependent the effects of fertilizing methods, how intensively they are influenced in soil by various factors — follow not only from consideration of the presented soil enzymatic results; the same fertilizing measure can influence the enzymatic activity of various soils quite differ-

ently. Enzymatic activity has a great importance in the turnover of fertilizing substances which are introduced into the soils. From the point of view of this concept we shall estimate the whole material electronically, here we could show only part of the results by means of some parameters. Only in this way can we get integrating results referring to the influence of cultivation measures on the soil fertility potences within several habitats.

SUMMARY

The effect of organic and mineral fertilizing have been investigated. By means of 5 permanent fertilizing experiments in different soils (2 heavy soils, 1 black earth, 2 light soils), in comparison a deficient variant (without fertilizing) was investigated too.

The effect of fertilizing was characterized by means of 4 enzyme activities (dehydrogenase, polyphenoloxidase, tyrosinase, catalase) which are compared with soil chemical parameters (total carbon content = C_t, colour quotient = Q 4/6, content of insoluble carbon = C_{rest}) and with the density of bacterial population.

In addition to yearly dynamic researches, results of yearly mixed samples are represented.

Concerning total carbon content colour quotient and density of bacterial population complete fertilizing (manure with NPK) effects high values; mineral fertilizing (NPK) induces lower values; in variants without fertilizing (deficient variants) the lowest values were established in each permanent fertilizing experiment (PFE).

From the enzymes only in case of dehydrogenase, complete fertilizing stimulates the enzyme activity more than mineral fertilizing; but the relation between the mineral fertilizing variant and the deficient variant is different in these experiments too. In case of all other investigated enzymes it is impossible to establish such a regularity; the feature is different for several enzymes.

It is impossible to draw a conclusion to fertility conditions of soil by means of a single soil chemical, soil biochemical or soil microbiological parameter.

The soil type conditions and habitat of soil complexly influence the effect of fertilizing measures. The effect and the process of the turnover of fertilizing material depend on numerous factors. This can be clearly shown by means of the different influences of fertilizing measures on the investigated parameters in different soil forms of PFE.

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ON THE ADSORPTION BEHAVIOUR OF BACTERIA IN THE SOIL

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Almost the entire effects, important for the formation of yields and soil fertility, e.g., conversion of humus and agrochemicals, are influenced to a high degree by the adsorption capacity of the soil. However, our knowledge is still very fragmentary in this respect. For this reason, we want to report here on a series of tests in which we have examined the influence of natural and industrially manufactured adsorbents on soil bacteria with different morphological and physiological characteristic in model tests.

In the centre of these complex problems the question of the process of the vital functions of the microorganisms in adsorbed state, was first considered which in many cases was found to be contradictory in the literature. (Müller and Hickisch 1969).

The following experimental programme was put into practice step by step:

We tested 12 different strains of bacteria and 9 different adsorbents (Table I).

Table 1
Experimental programme for adsorption tests

Bacteria	Characteristic	Adsorbents
1. *Pseudomonas fluorescens*	Cellular size Slime formation Movability Gram reaction Age of cultures	1. Quartz
2. *Bacillus brevis*		2. Kaolinite
3. *Arthrobacter simplex*		3. Montmorillonite
4. *Azotobacter chroococcum*		4. Bentonite
5. *Agrobacterium radiobacter*		5. Cation exchanger (Wofatit CP)
6. *Bacillus subtilis*		6. Anion exchanger (Wofatit N)
7. *Corynebacterium flaccumfaciens*		7. Adsorption resin (Wofatit EW)
8. *Bacillus cereus*		8. Adsorption resin (Wofatit EZ)
9. *Micrococcus sp.*		9. Loess
10. *Micrococcus flavus*		
11. *Brevebacterium helvolum*		
12. *Bacillus cereus var. mycoides*		

The basis of comparison was quartz.

From the group of the secondary clay minerals, kaolinite, montmorillonite, and bentonite were selected.

Wofatits are ionic exchangers on the basis of artificial resin which are industrially manufactured in the GDR to a greater extent and possibly may be valuable for future improvement of agricultural production areas with low adsorption capacity.

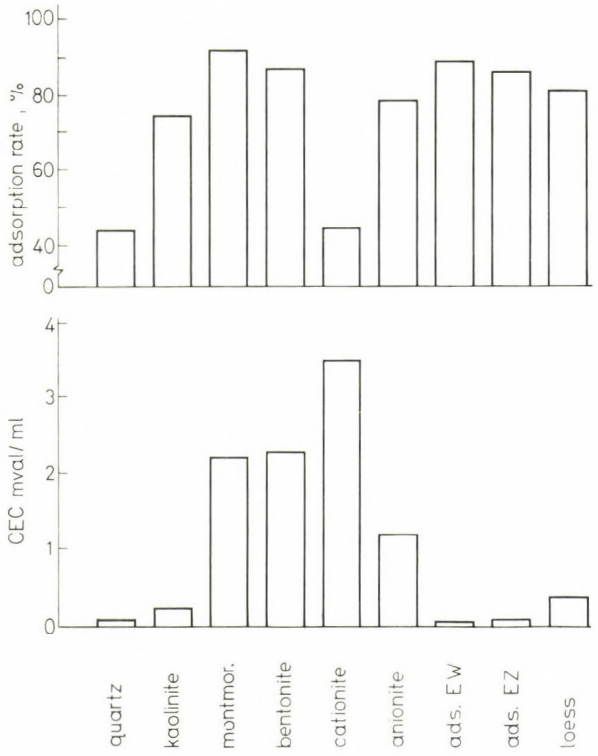
In the series of tests a cation exchanger (Wofatit CP), an anion exchanger (Wofatit N), and 2 adsorption resins (Wofatit EW and EZ) were involved.

As a first step to further investigations with soils, loess subsoil was included in the programme.

The results of tests on the inclination for adsorption indicated a specific influence of the bacterial strains, so that in further series of tests the influence of cellular size, slime formation, movability, Gram reaction, and age of the cultures on adsorption were investigated.

Figure 1 shows the ascertained rates of adsorption of the adsorbents in an average of all soil bacteria tested. Quartz, a stable mineral,

Fig. 1. Adsorption of soil bacteria on different adsorbents in comparison to the cation exchange capacity (CEC) of these substances



extremely neutral in its electrical charge, was only able to adsorb 44% of the offered cells, the smallest amount of all adsorbents tested. In contrast to this, kaolinite with a quadruply intensified ionic adsorption, had an adsorption rate for bacteria of 75%. Montmorillonite, a representative of the three-layer minerals, is, compared to kaolinite, marked in its surface tension by a higher amount of energy, evidenced by an exchange capacity eleven times higher than that of kaolinite.

For the adsorption experiments with bacteria, it was always our endeavour to obtain the same adsorption-active surface by use of a uniform grain size fraction. The inner surface of the three-layer minerals had not to be considered for adsorption of bacteria, since the distance of only a few Angström between the layers excludes any adsorption of bacteria, even of the smallest ones. In principle, also montmorillonite has the same effective surface like quartz and kaolinite. It is our opinion that by this fact the increase of adsorption of the bacteria by about 17% only, compared to kaolinite, may be explained. With 92% of adsorbed cells, montmorillonite obtained the highest value; the difference to kaolinite and quartz, however, was smaller than the cation adsorption in the soil which is of greatest importance for producing crops. Bentonite, another representative of the three-layer minerals, showed, as expected, a very similar behaviour to montmorillonite.

Also loess, with 82%, was able to retain a very considerable number of bacteria.

In comparison to these natural inorganic adsorbents just estimated, the industrially manufactured organic adsorbents on the basis of artificial resin showed much greater ability for ionic exchange.

Since the width of pores of the ionic exchangers is tested with ions of 30 Angström as a maximum, only the outer surface could be considered in adsorption tests with bacteria.

With 45%, the cation exchanger adsorbed practically only the same number of cells as quartz.

Under analogous conditions, however, the anion exchanger adsorbed about 79% of the cells offered.

This remarkable difference of 34% between the examined exchangers suggests a negative charge of the bacterial cells.

This presumption is further confirmed by the adsorption values of the resins Wofatit EW and EZ.

By reason of their structure, they both react like an anion exchanger; they were able to adsorb 87% and 90% of the offered cells, respectively.

In consideration of the indicated results, it seems difficult to explain the high adsorption rate of montmorillonite, since the negative charge of the bacterial cells and the surplus of negative charge by which montmorillonite is likewise marked seems to exclude an adsorption of cells on the basis of Coulomb attraction.

Here, above all, we presume that in systems with natural adsorbents pure substances may be present only to a restricted degree.

It is known that in the case of natural adsorbents, which certainly do not represent pure substances, accompanying substances may be dissolved which can neutralize negative charges both of the montmorillonite and of the bacterium or recharge them, (McCalla 1940).

Corresponding experiments with polyvalent cations in the suspension showed a distinct increase of the adsorption rate by polyvalent ions; with monovalent ions, the influence was within the range of methodology.

Having considered in some detail the great influence of the physicochemical properties of the adsorbents in adsorption experiments, now the influence of the bacteria and some of their morphological and physiological characteristics will be analyzed.

The adsorbents present in the samples with the same number of particles had been equalized to the approximate particle size of 2 μm; with the bacteria, however, only the number of cells in the suspension could be approximately equalized.

For the experiments, the role of the cellular size was therefore the first test factor, typical one of the species.

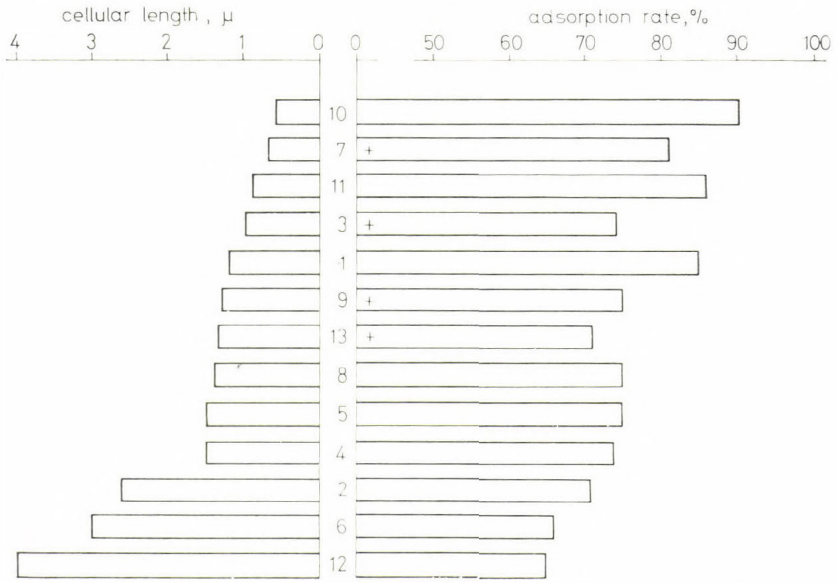
As shown in Fig. 2, the results give evidence that on the average all the adsorbents exhibit a decrease in their inclination for adsorption of the bacteria with increasing cellular size.

From *Micrococcus flavus* with a cell length of 0.66 μm and an adsorption rate of 90%, the adsorption decreases with increasing cellular length of the bacteria up to *Bacillus cereus var. mycoides* (length of cell 4.02 μm) to 60.5%.

The living cells, in dependence of their size, behaved like inorganic particles during the process of adsorption, however, by their slime formation they showed a specific behaviour, typical for the species, which concealed the influence of the cellular size.

Slime-forming bacteria were adsorbed to a less degree than those without slime formation.

Fig. 2. Adsorption of soil bacteria in dependence on their cellular length (+ strains with slime formation)



This is evidenced in Fig. 2, still more distinctly in Fig. 3, where strains of about the same cellular size, but of different ability of slime formation, are shown in relative proportion. The adsorption of the strains without slime formation was equal to 100%, compared to the strains with slime formation.

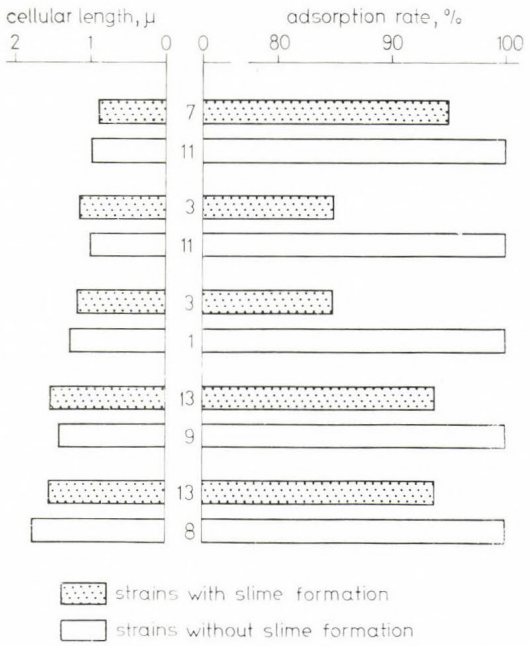


Fig. 3. Influence of slime formation on the adsorption of bacteria

As the cause of this decreased adsorption of the slime forming bacteria it was suspected first of all a negative charge of some bacterial slimes, proved in the experiments. In this case, however, the anion exchanger should not have shown a decrease of adsorption with the slime forming bacteria.

But also with the anion exchanger a depression was recorded. It was supposed that, for one thing, the decreased efficiency of the adsorbents may be caused aggregation of their smallest particles, induced by the slime, the result of which is a reduced surface; for another, that by increased viscosity of the suspension the cells might have been kept away from the adsorbent.

Also the movability of the bacteria is a property which should be taken into consideration in adsorption tests.

Active-mobile bacteria, as is well-known, are marked by several flagella on their surface.

For relevant tests, 2 strains of bacteria with flagella and 1 strain without flagella were selected and cultivated in liquid medium.

Following this, the cells of each of the strains were partly mixed with acridine orange and thus induced to throw off their flagella. Another part of the cells remained untreated.

After that the rate of adsorption was ascertained. The results were as follows:

Cells in a flagellated condition were more inclined for adsorption than cells of the same strain without flagella. The inclination for adsorption of the strain without flagella formation, both of the treated and the untreated sample, was the same. Thus, a diminution of the activity of the cells by treatment with acridine orange can be excluded. The recorded increase of adsorption is probably induced by the presence of flagella and their active movements.

The cause for this may be an enlarged surface of the cells, accompanied with an only slightly increased volume, however, also an active turning towards the adsorbent could be possible.

Adsorption experiments with bacteria of different Gram reaction resulted in contradictory findings hitherto, because the far-reaching uniformity of other essential characteristics was neglected.

For this reason, we selected 6 Gram negative and 6 Gram positive bacteria whose properties, considered as essential, conformed to a high degree.

The obtained results showed that the different Gram reaction of the 12 strains tested had no influence on the rate of adsorption.

The examination of the influence of the age of the bacteria on the adsorption showed the following results:

Of 6 strains of bacteria tested, which after growing at 27 °C for 24 hours were stored at 4 °C, the rate of adsorption decreased considerably only with 2 strains, and also with them only after 81 days.

However, the storing temperature was chosen the same as that of the incubation, the rate of adsorption decreased already after the 9th day.

Contradictory are the published experimental results concerning promotion or inhibition of the vital processes of the bacteria in adsorbed condition.

With our complex experimental series it was possible to ascertain the quantity of CO_2, evolved by the adsorbed cells only.

The results confirmed the findings of Stotzky (1966) and Novaková (1968) on the promotion of the biological activity by montmorillonite, furthermore also by loess and the adsorption resin EZ.

The CO_2 production of the samples increased from quartz over the synthetic cation exchanger kaolinite, the synthetic anion exchanger, the adsorbent resin EZ, loess, up to montmorillonite very much. This increase runs in its tendency parallel with the rate of adsorption.

Table 2 gives evidence of these facts.

Table 2

Comparison of the CO_2 production and the rate of adsorption of the bacteria, tested with different adsorbents

Adsorbent	CO_2, ml	Rel.	Ads., %	Rel.
Quartz	1.0	100	48.2	100
Cation exchanger	1.4	140	44.0	91
Kaolinite	1.7	170	77.1	160
Anion exchanger	1.8	180	87.0	180
Wofatit EZ	2.4	240	89.2	185
Loess	2.6	260	78.1	162
Montmorillonite	3.5	350	95.0	197

The adsorption increased from quartz (100%) over kaolinite, Wofatit EZ, and loess, and obtained with montmorillonite up to 197%.

In comparison to quartz, however, the CO_2 produced with montmorillonite was 350%.

By reason of these results, the occurrence of symptoms of depression with adsorbed microorganisms can be answered in the negative, it can even be referred to a considerable promotion of the CO_2 production, above all by montmorillonite and loess, but by the adsorption resin as well.

These are the most important results of an extensive investigation on the adsorption behaviour of bacteria with natural and industrially manufactured substances as adsorbents.

These results extend and deepen our knowledge in ascertaining the microbial processes in the soil which are of the greatest importance for the production of crop yields.

SUMMARY

In spite of the fact that almost all microbial processes affecting soil iertility are closely related with adsorption phenomena, our knowledge fn this field is inadequate, first of all as to the vital processes of bacteria in adsorbed state.

We carried out a series of model experiments and applied to them 12 strains, as to their morphological and physiological properties, different bacterial orgins and 8, partly natural and partly (for purposes of the industry made) artificial adsorbents.

As a first approach to the solution of the complex problem we investigated the adsorption capacity of bacteria, furthermore the influence of cell size, slime formation, mobility, Gram reaction as well as the age of bacterium cultures upon the adsorption to various adsorbents.

The results obtained have been discussed.

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THE ROLE OF SOIL MICROORGANISMS IN THE TRANSFORMATION OF ORGANIC PHOSPHORUS IN SOIL

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The elucidation of the specific role of soil microorganisms in transformation of phosphor-organic compounds is an important task of soil microbiology. The study of their enzyme activities and phosphorus metabolism in micro-organism — soil — plant complex system has the most perspective both from a scientific and practical point of view.

Nuclease and phosphatase activities of soil bacterial populations and the transformation of different native and soil phosphor-organic compounds in connection with phosphatase and nuclease activities of soils were studied.

MATERIAL AND METHODS

Nuclease activity of bacteria was determined by the acid precipitation method of Jeffries et al. (1957); the enzymic soil activity was studied using our method (Burangulova and Chasiev 1965); phosphatase activity of bacteria colonies was determined by using sodium phenolphthalein-phosphate; organic phosphorus fraction — suggested by Heyfets (1948).

It was found that many groups and species of soil bacteria have the ability to produce the enzymes of nuclease and phosphatase (Table 1).

Table 1

Nuclease and phosphatase activities of bacteria isolated from a grey forest soil

Enzymes / Bacteria	Ribonuclease			Desoxyribonuclease			Phosphatase		
	Tested	No.	% Active	Tested	No.	% Active	Tested	No.	% Active
Bac. agglomeratus	2	0	0	—	—	—	—	—	—
Bac. mycoides	11	0	0	—	—	—	5	0	0
Bac. adhaerens	35	2	5.5	7	0	0	7	3	42.8
Bac. cereus	25	21	84.0	9	6	66.6	10	9	90.0
Bac. glutinosus	17	1	5.9	6	0	0	0	0	0
Bac. megatherium	21	17	80.9	4	0	0	4	1	25.0
Bac. mesetericus	17	15	84.1	7	2	18.5	7	3	42.8
Pseudomonas	53	5	9.4	25	1	4.0	46	20	43.5
Mycobacterium globiforme	32	4	12.5	6	0	0	6	1	16.6

One has to remark that the ability of bacteria to produce nuclease and phosphatase extracellular enzymes is not a constant characteristic. It varied according to the different strains of the same species.

According to their enzyme activities one can distinguish the following bacterial groups: of the investigated: 1) bacteria with slight nuclease and phosphatase activities *(Bac. glutinosus, Bac. mycoides*, the most bacteria — *Mycobacterium* and *Micrococcus)*; 2) with high phosphatase activities *(Bac. adhaerens, Pseudomonas)*; 3) with rhibonuclease and phosphatase activities *(Bac. cereus, Bac. megatherium, Bac. mesentericus)*; 4) with DNA — asic, RNA — asic and phosphatase activities *(Bac. cereus, Bac. subtilis, Bac. mesentericus)*.

Analyzing the relative contents of bacteria according to mentioned groups it will be noted that most representatives of bacterial populations of grey forest soils have slight nuclease activity and are not able to decompose high-molecular phosphor-organic compounds. This fact has apparently great ecological significance in the physiological activity of microorganisms in different types of soils. In grey forest soil microorganisms apparently use primarily mobile mineral phosphorus compounds and low molecular phosphororganic compounds, also, having free phosphorus group which can be lightly chipped off by phosphatase. The formation of extracellular nuclease in this case is not vitally necessary for them.

Quantitative bacterium distribution which can split highly polymerized nucleic acids *(Bac. megatherium, Bac. mesentericus, Bac. subtilis)* also depends on soil conditions. In non-chernozem soils they are found in very negligible numbers (Table 2). In chernozem soils these species are widely distributed which is connected with their ability to assimilate nitrate nitrogen and highly polymerized organophosphoreus compounds which dominate in the soils.

Table 2

The occurrence of spore-forming bacteria in the soils (in %)

Soil	*Bac. agglomeratus*	*Bac. mycoides*	*Bac. adhaerens*	*Bac. cereus*	*Bac. glutinosus*	*Bac. megatherium*	*Bac. mesentericus*
Light grey	17.4	0.8	24.6	8.0	17.4	1.2	0.4
Grey	12.1	1.8	9.2	3.3	12.3	1.5	1.4
Dark-grey	16.5	0.9	6.9	5.8	14.9	0.9	0.0
Podzolic chernozem	3.1	0	0	3.1	0.8	31.1	14.2
Leached chernozem	0.5	—	1.6	1.6	0	18.5	11.1

As it is shown in Table 2 in the chernozem soils from the bacterium groups which are most able to mineralize with high molecular weight organophosphorous compounds the representatives of *Bac. megatherium, Bac. mesentericus* groups, occurred and in grey forest soils the representatives of *Bac. cereus* can be found. The wide distribution of the latter in the grey forest soils is due to the fact that they are not able to assimilate mineral nitrogen and at the same time in the given soil conditions they have very high nuclease and phosphatase activities exceeding *Bac. megatherium* and *Bac. mesentericus* activities. In grey forest soils with adequate organic

nitrogen content the presence of active nuclease of *Bac. cereus* has advantages over other bacterium groups.

The data prove that on biochemical mobilization of organic and plant residues it is necessary to have different approaches taking into consideration soil conditions and content and enzymic activities of microbic cenosis.

In close connection with microbe population having phosphatase and nuclease activities there are the corresponding enzymic activities of soils. In contrast with grey forest soil phosphorus metabolic processes proceed more intensively in chernozem. This is due to its greater population by phosphormineralizing and in this connection by comparatively high phosphoesterase activities of soils themselves.

As Table 3 illustrates the intensity of dephosphorylation of organophosphorous compounds in soil correlates with the activities of corresponding enzymes.

Table 3

*Phosphoesterase activities and dissociation of organo-phosphorous compounds in the soils**

Soil	Organic phosphorus			Enzyme activities		
	Nucleate-Na	Phytate-Na	Glycero-phosphate-Na	Nuclease	Phytase	Glycero-phosphatase
	in % to substratum			mg P_2O_5 per 100 g soil		
Grey-forest	11.96	—	43.94	5.98	—	21.97
Chernozems:						
Leached	15.95	4.96	77.20	7.98	2.48	38.60
Typical	18.62	3.65	83.22	9.24	1.82	41.61
Calcareous	32.98	1.98	80.43	16.48	0.99	40.20

* The concentration of organo-phosphorous compounds was equivalent to 50 mg P_2O_5 per 100 g soil each, incubation time: 24 hours.

The speed of mineralization of organic phosphates depends also on their chemical peculiarities. In the experiments native organic phosphate composting for 80 days with the leached chernozem in optimal hydrothermal conditions it was observed that low-molecular organo-phosphates as a result of their non-specific hydrolysis by different phosphatases hydrolyze almost wholly and do not accumulate in soil notably (ATP, glycerophosphates, sugar phosphates (Table 4). Nucleic compounds and phytates as a result of their high specificity with respect to hydrolytic enzymes and lower activities of corresponding enzymes in soils dissociate less intensively and accumulate in the soil. That is why most of the soil organic phosphorus is apparently present by these compounds (Wrenshall and Dyer 1941, Fabry 1960, Burangulova 1960). Consequently the measures for improving the phosphoric regime of soil at the expense of soil supplies of organic phosphates must be directed primarily at mobilization of stable nucleic and phytinic phosphates of organic soil complex.

The transformation of organic phosphate of soils themselves and its dependence on enzyme activities is of great interest.

Table 4

The intensity of dephosphorylation of the native organic phosphates in soil

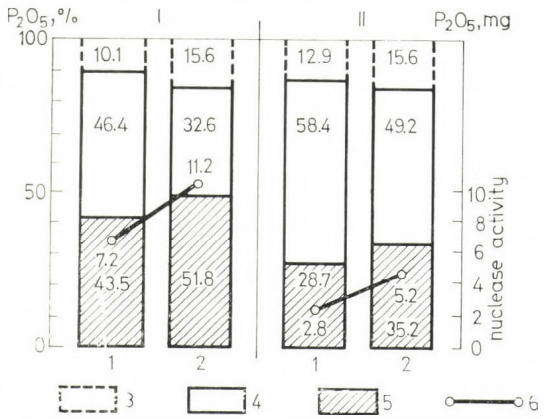
Variant	Organic phosphorus extracted from soil	Mineralization of applied phosphorus	Dissociation of organic phosphorus applied in soil in %
	mg P_2O_5 per 100 g soil		
Control	102.9	—	—
Nucleate—Na	124.9	27.50	55.0
RNA	111.3	21.57	33.1
Glycerophosphate-Ca	103.9	49.02	98.0
Phytyn	145.2	7.70	15.4
ATP	104.9	47.98	95.9

During a month the composting of calcareous and leached chernozems was carried out and the changes of correlation of acid-soluble and alkaline-soluble fraction of organic phosphates and soil nuclease activities were observed (Fig. 1).

The interdependence between nuclease activities changes and the mineralization of ammonia-soluble fraction of organic phosphorus like nucleic compounds is observed.

Apparently, the labile fractions of soil nucleic acids are subjected primarily to biochemical mineralization. Analogous changes of organic phosphorus content on the composting of chernozems were also observed by Fabry (1963), however, the author does not connect dynamics of organic phosphorus with the changes of enzymic activities.

Fig. 1. The change of organic and mineral phosphates content (% to sum) and soil nuclease activity during composting. I calcareous chernozem; II leached chernozem. 1) soil before composting; 2) after composting: 3) mineral phosphorus; 4) ammonia-soluble organic phosphorus; 5) acid-soluble organic phosphorus; 6) nuclease activities



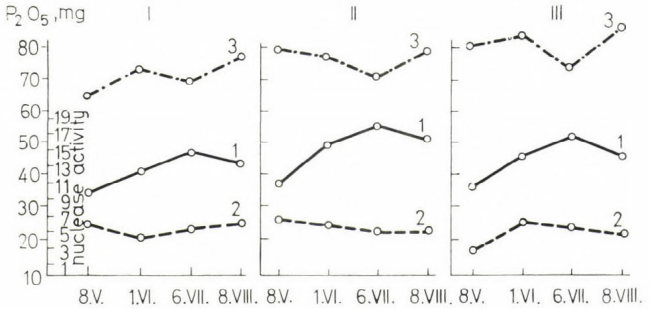


Fig. 2. The dynamics of organic phosphates content and nuclease activity in calcareous chernozem. I control; II manure; III NPK; 1) nuclease activities; 2) acid-soluble organic phosphorus; 3) ammonia-soluble organic phosphorus

In connection with higher activities of nuclease and lesser stability of nucleic acids their mineralization in calcareous chernozem proceeds more intensively (14%) than in leached one (9%).

In field experiments there was a positive correlation between nuclease activity of the soil and the amount of ammonia-soluble organophosphorous compounds, as it is shown in Fig. 2. The investigation was done on the control plots, fertilized by manure (20 t/ha) and full mineral fertilization ($N_{45}P_{60}K_{45}$) during the vegetative period. As shown in Fig. 2, the seasonal content change of ammonia-soluble organic phosphorus has an opposite tendency to the dynamics of nuclease activities. The hydrolytic dissociation of the above mentioned forms of organic phosphates is connected with the action of soil nuclease.

SUMMARY

Nuclease and phosphatase activities of microorganisms are determined by soil conditions. Intensity of the hydrolysis of native and soil organic phosphates depends on enzymic activity of microflora and soil. The activities of phosphoesterases indicate the intensity of biochemical mobilization of different soil organo-phosphorous compounds.

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ON THE PROBLEM OF MODELLING IN SOIL ENZYMOLOGY

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The development of soil enzymology is characterized at present by investigations on single enzyme reactions and on those occurring in soils. But "dismembering the chemical continuity of processes... we cannot omit our obligation to restore the disintegrated parts in their wholeness"according to this sentence of the eminent biochemist Lipmann (1948, p. 57) is quite pertinent in defining the tasks of soil enzymology. Now we have to simulate processes integrating substantial and energetic metabolism performed in soil.

In the soil both reactions occur due to enzymes of various origin and directed by vectorial processes being affected by the totality of enzymes and surrounding organisms, as also by the synthesis of substances like humus, amino acids, etc.

Modelling may be considered as one of the ways of elucidating the role of living single components in the formation of enzymatic level of the soil. The importance of modelling in natural and scientific experiments is now generally accepted. The realization of models in biology enabled the solution of a series of essential biological problems, particularly in biochemistry. The main advantages of such models in biology consist in their ability to simulate natural biologic systems, deliberately simplifying and schematizing them, which stimulates a better understanding of the main point of the phenomenon (Beament 1963).

An example of a biological model is the artificial percolation which creates conditions simulating natural ones, in the sample of soil flow and also by contamination of sterile soil samples by single components of edaphon followed by activity determination of some enzymes. These kinds of experiments enable the solution of the problem of the origin of soil enzyme and specific role of single edaphon components in the formation of a natural enzymatic soil level.

As we have shown, the enzyme activity of single edaphon components may be higher than in the soil itself from which they have been removed (Kozlov and Niucheva 1968). For instance, the activity in a culture of *Pseudomonas sp.* isolated from salt-marshes of the SE Transbaikalian region, the redoxases activity (polyphenoloxidase and peroxidase), exceeded (by 29—44 per cent) the activity in these soils.

To elucidate the role of single edaphon components in the formation of a natural enzyme level of the soil we inoculated previously sterilized soils with pure organism cultures (mainly with fungi) or their mixtures, isolated from the same soils. The soil after preliminary autoclave treat-

ment was maintained at 60 per cent humidity as based on full moisture capacity, at +25—27 °C for 7 days.

Two soils have been applied, a leached chernozem and a soddy soil from the southern part of the Irkutsk region. The cultures were isolated from the chernozem soil. The results of these experiments are summarized in Table 1.

Table 1

Enzyme activity on a soil inoculated by fungi (to 1 g of soil)

Culture	Catalase		Saccharase		Peroxidase		Polyphenoloxidase	
	I	II	I	II	I	II	I	II
Penicillium sp.	0.27	0.61	16.8	14.7	2.8	4.5	0.6	1.6
Trichoderma lignorum	0.10	0.45	15.63	13.4	1.6	3.2	0.2	1.1
Fusarium sp.	0.2	0.88	15.1	10.5	2.4	4.0	0.8	0.4
Actinomyces griseus	—	1.08	—	13.4	2.0	—	0.5	—
Penicillium + *Trichoderma*	0.27	0.50	15.3	14.4	1.6	15.2	1.2	5.9
Fusarium + *Actinomyces*	0.34	0.27	0.7	2.7	4.8	1.6	0.8	0.4

Note: I—chernozem, II—soddy soil — no experiment performed

The above-mentioned data show that enzyme activity in experimental soils exceeds the activity determined in natural soil, used as control. Therefore, the contamination of sterile soils with pure fungi cultures or their mixtures promotes such an activity of single enzyme or approaches the characteristic level for a natural control or exceeds the latter.

In order to elucidate this problem in detail we performed sets of corresponding experiments with percolators (Kislitsina 1966, Markova 1967). We have obtained the following data for the activity of cellulase (1) (Table 2).

Table 2

*Effect of fungi on cellulase activity of the chernozem of SE-Transbaikalian region**

Experimental conditions	Activity, 1 μg of glucose to 1 g of soil or 1 ml of filtrate	Reduction as compared with reference, per cent
Air-dry soil as reference	28.0	—
Soil treated in an autoclave	22.4	100
Penicillium sp.	4.0	—
Dematium hispidulum	9.2	—
Autoclaved soil + cultural liquid *Penicillium*	13.2	59
Autoclaved soil + cultural liquid *D. hispidulum*	12.6	56

* The technique of cellulase activity determination has been reported in an early publication (Kislitsina, Kozlov, 1968; Kislitsina, 1966; Kozlov, Kislitsina, 1967).

It has been shown that soil inoculation by any species of fungi displayed a cellulolytic activity totalling 55—59 per cent of the initial activity

of the autoclaved chernozem soil. Thus autoclaving treatment and inoculation with some fungi culture promotes a sufficiently high level of cellulase activity.

We have obtained similar data in a study on nuclease (RNA-ase) activity (Markova 1968).

At perfusion through an oven-heated chernozem soil of SE-Transbaikalian region with cultures of *Bacillus megatherium* or *Penicillium citrinum* and a high content of sodium nucleate as a substrate, the activity of RNA-ases exceeded the initial by 55.5 per cent or equalled it correspondingly. These data convincingly support the important part played by microflora in the formation of enzyme level in soils (Table 3).

Table 3

Effect of organisms on RNA-ase activity of chernozem soil

Experimental conditions	RNA-ase activity mg of P_2O_5/1 g soil	Reference, per cent
Air-dry soil as reference	0.007	100
Heated soil	0.0	—
Heated soil + *B. megat.* + nucleate	0.046	657
Heated soil + *Pen. citr.* + nucleate	0.007	100

The study of phosphohydrolase activity in cultures of organism isolated from East-Siberian soils has shown this activity to be significantly higher than in the soil. For instance, the endophytase activity of *Penicillium citrinum* equalled 1.73 mg P_2O_5/1 g dry mycelium and in the soil from which it has been isolated 0.008 mg P_2O_5/1 g dry soil (Markova 1968).

The above-mentioned data show unambiguously that the activity of single enzyme components of edaphon may be higher or lower than the activity level in the edaphotope (soil). The quantitative evidence of this activity depends on the given medium conditions.

Application of a computer (BCEM) to reveal medium factors determining the soil enzyme activity (namely, proteases) has shown that this activity depends a great deal on the resources of humus and mobile nitrogen forms ($+ = 0.40 - 0.59$) and also on the presence of producing forms for the given group of enzymes (ammonifying bacteria, sporulating forms of bacteria, fungi and actinomycetes). The correlation coefficient in this case has been $+0.74$ (Mikhailova 1969).

All this indicates the significant part played by the microflora in soil enzymatic activity formation.

Since root systems of higher plants also contribute to the biological soil activity, the latter has been determined by us near the roots also. Mean values of this experimental work are summarized in Table 4.

The activity of root system enzymes of agricultural plants is fairly high and may exceed both activities, of soil and of microflora isolated from it.

During the last years much attention has been paid in biology to the soil fauna as a constitutive part of soil population (Gilyarov 1965). The enzymatic activity of this edaphon component has been hardly studied

Table 4
Enzymatic activity of higher plant root systems (based on 1 g of dry root mass)

Plant	Catalase, mg H_2O_2	Saccharase mg. inv. sugar	Peroxidase	Polyphenol-oxidase
			iodine, ml	
Corn	9.5	73.7	2.0	40.0
Bean	8.3	100.6	4.0	24.0
Wheat	5.1	65.3	1.6	16.0

comparatively (Ukhtomskaya 1952, Antoniani et al., 1954, Kiss 1957, Hoffmann 1959, Kozlov 1963, 1965).

The enzymatic activity being related to degradation and synthesis of organic matter, we estimated the oxidase activity (polyphenoloxidases, peroxidases, catalases and dehydrogenases) and cellulases in soil and extractions of the investigated soil fauna (Kozlov 1965, Kislitsina 1966). Data obtained by these determinations are presented in Table 5.

Table 5
Enzyme activity in soil, matter from ant-hills and extract obtained from the soil fauna (1 g soil or substrate)

Enzyme sources	Dehydrogenase, mg, TFF	Catalase mg H_2O_2	Polyphenol-oxidase	Peroxidase
			iodine, ml	
Soil from ant-hill, *Formica fusca*	0.006	24.6	6.8	9.9
Young working ants	0.106	32.1	3.1	13.4
Chrysices-ova of ants	0.157	10.8	8.2	9.6
Soil near the ant-hill	0.002	2.5	0.4	2.6
Soil from the ant-hill, *Formica rufa*	0.003	17.5	5.6	8.1
Ants' chrysices	0.054	6.8	1.18	3.4
Soil near the ant-hill	0.001	5.1	3.7	5.2
Rainworms *Lumbricus terrestris*	0.125	29.7	1.38	2.7
Soil inhabited by worms	0.021	27.2	1.29	2.9
Grubs of may-cockchafers *Meldlonthia hippocastanea* F.	0.024	25.1	11.8	10.2
Grubs of bronze crackers *Selatosomus alilus* Z.	0.032	5.1	1.31	4.1
Soil inhabited by insects	0.005	28.5	10.3	9.7

As shown by the table, oxidase activity reaches higher levels in representatives of soil fauna than in soils inhabited by them. Some differences in activity of this group of enzymes may be observed for single representatives of fauna due to specific particularities and other medium factors.

Analogous data are obtained for cellulase. Thus for ant *Formica uralensis* the cellulase activity has been shown to be 30 and for *Companatus herculanus* 31 μg glucose/1g, respectively.

Investigations of other fauna representatives characteristic for "taiga" landscapes of Eastern Siberia (in particular, caterpillars of the Siberian

bombyx, *Dendrolimus sibiricus* Tschetv.) have also shown a higher activity of oxidases as compared with the cedar needles and soil of their inhabitation ones (Kozlov 1965). This demonstrates the important part played by fauna in soil enrichment by enzymes.

It is impossible at present to determine the specific weight of soil fauna in the biological activity formation of soil but the data offered prove it without doubt.

As shown by model experiments, the biological activity of soil (measured by activity of some enzyme or their totality) represents not simply the sum of enzyme activity, as produced by some single edaphon components (organisms, soil fauna, higher plants roots). It differs as a rule from the activity value, being higher or lower than it. This activity of single edaphon components is being determined by concrete environmental conditions, type of the soil and its genesis, composition of biocenosis and the complex of abiogenous and biogenous factors, characteristic for the particular soil (Kozlov 1968).

We consider the modelling, i.e. inoculation of soil or its analogues by representatives of soil population in percolatory experiments followed by treatment of data obtained with computers, as a means of elucidating the specific weight of single edaphon components in enzymatic (biological) activity formation of soil.

This trend seems to have good prospects in the future for the whole of biology as it may serve to establish the laws and special relations existing in soils.

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EFFECT OF ORGANIC SUBSTANCES ON NITRITE FORMATION BY NITROSOMONAS

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Nitrification in soil is of great importance from the point of view of fertility of land. Since the discovery of the process of nitrification by the French workers Schloesing and Muntz, in 1877 and the isolation of the micro-organism, *Nitrosomonas*, by Winogradsky in 1899, a large amount of work has been done to have a clear picture of the whole process of bacterial nitrification in soil, but still the position is not quite clear. There have been different and contradictory opinions about the effect of organic substances on growth and activity of *Nitrosomonas*. It was reported by Warington (in 1878—1884) and the pioneer microbiologist Winogradsky in 1899 that many organic materials, such as glucose and other sugars are toxic to the nitrifiers. In 1936 Pandalai and later many others made similar observations. Gundersen (1955) has shown that although certain amino acids are toxic to the nitrifying bacteria, a few vitamins do not produce any inhibitory effect on the process of nitrification by *Nitrosomonas*. On the other hand, in 1956 Ruban reported that although the presence of large amounts of organic substances stopped the process of nitrification, the organism did not get killed and resumed activity when transferred to a fresh culture medium.

Thus, it can be realised that there were two different and contradictory opinions about the effect of organic substances on the growth and activity of *Nitrosomonas*, one suggesting toxic effect while the other an inhibitory effect. The problem therefore, needed a more detailed study.

In view of the fact that sugars and other organic substances serve as food materials for most living beings, this seems quite unconvincing that organic substances are toxic to the nitrifiers and their presence inhibits the process of nitrification. I along with my students, therefore, studied in detail the effect of sugars and some other organic compounds on the growth and activity of *Nitrosomonas*. The compounds taken for this study were glucose, fructose, mannose, galactose, xylose, arabinose, lactose, maltose, mannitol and tartaric acid.

Isolation of Nitrosomonas. A pure culture of the nitrifier, *Nitrosomonas*, was prepared from the garden soil by Omeliansky's method of elective culture in conjunction with the silica gel method of Winogradsky.

Effect of glucose on nitrification. We used different amounts of glucose for a definite quantity of the medium containing *Nitrosomonas* and estimated the amount of glucose used up at different intervals of time. In another set of experiments we estimated colorimetrically by Duboscq colorimeter, the amount of nitrite formed at different intervals of time in presence

of different amounts of glucose as well as in the absence of glucose. We also estimated the amount of total nitrogen present in the medium at the beginning of the experiment and at the end of the experiment and found that there occurred no loss in the nitrogen content of the medium during the course of the experiment.

Our observations (Tables 1, 2 and 3) and conclusions are as follows:

Table 1

Nitrification in the presence of D-glucose

Volume of the culture medium taken = 80 ml
Volume of the enriched culture (inoculum) added = 1 ml
Volume of the ammonium sulphate (10% solution) added = 1 ml

No.	Amount of D-glucose added to the medium (in mg)	Amount of D-glucose left at different intervals of time						
		Time in hours						
		48	96	144	192	240	288	336
1	Control	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	5.00	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3	10.00	4.37	Nil	Nil	Nil	Nil	Nil	Nil
4	20.00	10.94	4.37	Nil	Nil	Nil	Nil	Nil
5	25.00	17.50	8.75	3.64	Nil	Nil	Nil	Nil
6	30.00	19.68	12.39	5.10	Nil	Nil	Nil	Nil
7	40.00	29.16	24.06	9.45	2.92	Nil	Nil	Nil
8	50.00	34.26	28.43	9.45	2.92	Nil	Nil	Nil
9	60.00	48.84	45.20	27.70	10.93	3.64	Nil	Nil
10	80.00	71.44	67.80	50.30	29.89	16.04	6.56	Nil
11	100.00	97.69	92.58	71.44	52.49	40.09	19.68	5.10

Control = containing no glucose.

Table 2

Nitrification in the presence of D-glucose

Volume of the culture medium taken = 80 ml
Volume of the enriched culture (inoculum) added = 1 ml
Volume of the ammonium sulphate (10% solution) added = 1 ml

No.	Amount of D-glucose added to the medium (in mg)	Nitrite formed at different intervals of time (mg/l)						
		Time in hours						
		48	96	144	192	240	288	336
1.	Control	2.85	5.31	8.84	38.34	69.00	141.54	241.46
2.	5.00	—	3.41	6.90	33.12	88.46	172.56	270.62
3.	10.00	—	2.21	4.60	24.15	67.90	164.26	324.02
4.	20.00	—	—	2.30	10.45	28.75	74.43	230.00
5.	25.00	—	—	1.56	7.08	24.15	57.50	219.05
6.	30.00	—	—	1.56	4.44	19.17	53.04	153.36
7.	40.00	—	—	—	2.12	10.45	28.75	104.55
8.	50.00	—	—	—	1.11	6.79	10.45	57.50
9.	60.00	—	—	—	—	1.84	9.20	46.00
10.	80.00	—	—	—	—	—	3.68	18.55
11.	100.00	—	—	—	—	—	—	4.60

Control = containing no glucose.

Table 3

Nitrification in the presence of large concentrations of D-glucose

Volume of the culture medium taken = 80 ml
Volume of the enriched culture (inoculum) added = 1 ml
Volume of the ammonium sulphate (10% solution) added = 1 ml

No.	Concentration of D-glucose		Nitrite formed in 840 hrs mg/l
	Amount of D-glucose added to the medium (in mg)	Amount of D-glucose left after 840 hrs (in mg)	
1	Control	—	848.40
2	100.00	—	836.90
3	200.00	—	810.00
4	300.00	58.00	—
5	400.00	212.00	—
6	500.00	367.00	—
7	600.00	516.00	—
8	800.00	742.00	—
9	1000.00	962.00	—

Control = containing no glucose.

DISCUSSION

The results clearly showed that no nitrite was formed during the first 48 hours after the addition of 5 mg glucose. Later after 96 hours the rate of nitrite formation gradually increased. With 10 mg of glucose a similar result was obtained, and after 336 hours the amount of nitrite formed was even more than that with 5 mg. With different amounts of glucose up to 100 mg it was found that nitrite formation in every case did take place although the time lag for the nitrite formation to begin increased as the amount of added glucose increased.

Thus, the presence of glucose in small concentrations hindered the process of nitrite formation in the beginning for a certain period and during this period the glucose concentration went on decreasing. After this period, when the glucose concentration became negligibly small, the nitrite-formation began and took place even more vigorously than when no glucose was added; and it can be seen from the tables that during this period there was no glucose present in the medium.

In the absence of organic substances (control) the bacteria derive energy for their growth and metabolism from the oxidation of ammonium salts to nitrite and therefore, the nitrite-formation takes place from the very beginning. But, in the presence of organic substances they preferentially utilize the organic substances and derive energy from the oxidation of these substances. Since under this condition, the energy requirements of the organism are fulfilled from organic compounds, it does not need to oxidise ammonium salts to obtain energy, and hence the nitrite formation does not take place. It is only when the organic substances have been consumed that the bacteria begin to oxidize the ammonium salts to obtain energy needed by them. It is quite obvious from the graph

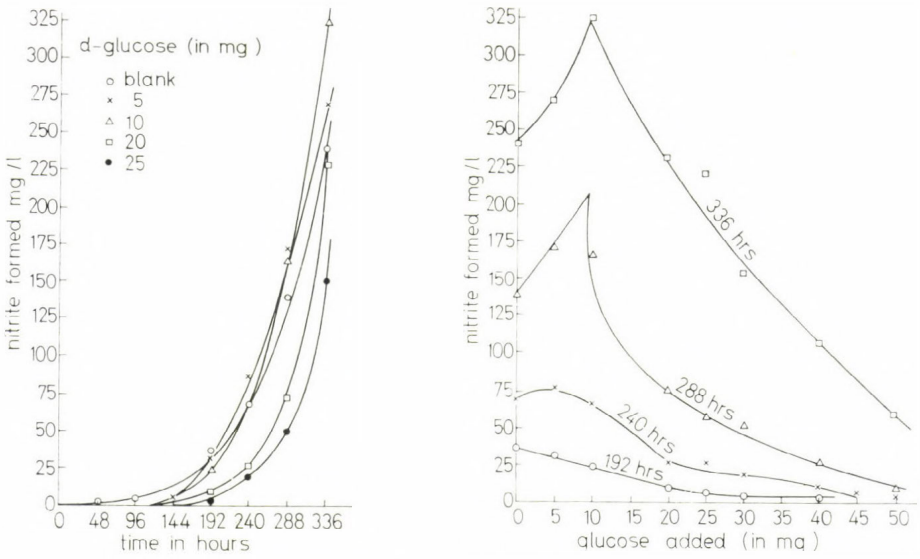

Fig. 1. Nitrification in the presence of D-glucose

(Fig. 1) that the nitrite formation goes on increasing in the later stages in each case. As is clear from the graphs and from the tables, the higher concentration of nitrite found in the sample containing 5 mg or 10 mg of glucose after 288 and 336 hrs as compared to the sample containing no glucose (control) can only be interpreted on the basis that glucose stimulates the growth and multiplication of bacteria to a larger extent, as a result of which the sugar containing culture solution comes to have a larger number of bacteria as compared to the sample with no sugar. Consequently, after the sugar has been consumed this solution, because of the larger number of bacteria present in it, shows a more vigorous nitrite formation.

Thus, we are led to conclude that glucose is not toxic or harmful to *Nitrosomonas* when supplied in small amounts (optimum amount).

We also studied nitrification in the presence of larger concentrations of D- glucose. It is clear from Table 3, that as the concentration of glucose is increased, the nitrite formation is not only delayed but is also retarded. With 1000 mg of glucose per 82 ml of the medium, the amount of glucose consumed by the bacteria even after 840 hours is only about 38 mg; with 800 mg of glucose the consumption is 58 mg, with 600 mg of glucose the consumption is 84 mg and with 500 mg the consumption is 133 mg and so on. Thus, it can be seen that as the initial amount of glucose taken is decreased, the rate of consumption of glucose increases and accordingly nitrite formation starts earlier. The rate of nitrite formation also shows an increase with the decreasing amount of glucose taken initially. It can, therefore, be concluded that higher concentrations of glucose are harmful to the bacteria. However, it has been found by us as well as by Ruban (1956) that the bacteria are not killed in the presence of higher concentrations

of cgucose. It is thus clear that in the presence of larger amounts of glulose, it is only the activity of the bacteria that is affected. This fact, too, is not surprising as other living organisms including the higher ones need only a limited amount of nutrients, and the food material taken in quantities much in excess of requirements produces ailments and sometimes may even cause death. It, therefore, seems quite reasonable that although glucose serves as a food material for *Nitrosomonas*, if taken in excessive amounts it produces harmful effects and depresses the growth and activity of the organism. Kalinenko (1953) showed that the presence of organic food materials in the culture medium of *Nitrosomonas* increases the bacterial proteinous mass. This observation too lends strong support to our view that simple non-toxic organic food materials instead of causing any toxic effect produce beneficial effect on *Nitrosomonas* provided they are supplied in proper amounts.

I have discussed the results obtained with D-glucose. The results obtained with other sugars and with mannitol and tartaric acid were similar to those obtained in the case of glucose. However, the optimum amounts of compounds to produce enhanced growth of bacteria and the time required to produce higher concentrations of nitrite may vary with different compounds.

SUMMARY

1. Nitrification by *Nitrosomonas* in the presence of sugars (glucose, fructose, mannose, galactose, xylose, arabinose, lactose and maltose): — The smaller concentration of sugars produces beneficial effects over the growth of *Nitrosomonas;* they hinder the process of nitrite formation in the beginning for a certain period after which the nitrite formation begins and takes place even more vigourously than when no sugar is added. The larger concentration of sugars although retards the process of nitrification, yet the bacteria are not killed.
2. Nitrite-formation by *Nitrosomonas* in the presence of mannitol and tartaric acid takes place in the same way as in the presence of sugars.

On the basis of our experimental results we are led to conclude that sugars and other organic materials are not toxic or harmful to *Nitrosomonas* when supplied in proper amounts (optimum amount). In the absence of organic substances, the bacteria derive energy for their growth and metabolism from the oxidation of the ammonium salts to nitrite. But in the presence of organic substances, they preferentially utilize the organic substances and derive energy from the oxidation of these substances. Since under this condition the energy requirements of the organism are fulfilled from organic compounds, it does not need to oxidise ammonium salts to obtain energy and hence the nitrite formation stops. It is only when the organic substances have been consumed that the bacteria begin to oxidise ammonium salts for obtaining the energy needed by them. The observed increase in nitrite content in the latter stage clearly indicates the enhancement in growth and activity of the organism in the presence of organic substances.

When present in large amounts, although these substances do stop the process of nitrification, yet the bacteria are not killed. This fact, too is

not unexpected, as other living organisms including higher ones need food materials only in a definite quantity, and the food material taken in quantities much in excess often produces ailments and sometimes may even cause death. It, therefore, seems quite reasonable that although sugars and other organic substances undoubtedly serve as food materials for *Nitrosomonas*, yet, if they are taken in excessive amounts they produce harmful effects and depress the growth and activity of the organism.

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SOME PROPERTIES OF ENDOGENOUS DISSIMILATORY NITRATE REDUCTION

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The research on the processes due to the effect of the biological dissimilatory nitrate reduction — which are harmful from an agricultural point of view — has two trends. Firstly, with the investigations of the nitrogen economy of the soil, the losses caused by denitrification can be measured. Secondly, by studying the microorganisms taking part in the process, those conditions which limit the biological activity of denitrification can be revealed.

Research on the physiological characteristics of organisms responsible for this process has indicated that advantageous environmental conditions for denitrification processes could develop in the rhizospheric zone of the plant and especially in the perennial herbaceous plants. This may take place because of the low oxygen tension caused by the metabolic processes of the plant and microorganisms living in the rhizosphere. Moreover, the plants excrete compounds which are very well utilized by some bacterial species, such as in their nitrate-reduction processes.

Naturally, the characteristics of the dissimilatory nitrate reduction cannot be observed by investigating the physiological properties alone. These processes can be evaluated only in accordance with results from biochemical and synecological research.

The starting point of our examination was based on the results of Woldendorp (1963). He has shown that among the species having capabilities for dissimilatory nitrate reduction, and which can be isolated very often from the soil such as *Bacillus licheniformis* and *Pseudomonas stutzeri*, can produce gas from nitrate. The former produces ammonia, the latter nitrite and NO_2. He proved also that the biochemical mode of the dissimilatory nitrate-reduction was influenced by the quality of the C-sources and that the nitrate and nitrite-reduction within one bacterium species took place in the presence of various electron donors in different ways.

When we began to study these problems it appeared that the washed cells of the above-mentioned organisms could reduce a significant amount of nitrate under anaerobic conditions, without the additions of electron donors. Considering that we could not find data on the anaerobic endogenous nitrate reduction, we conducted our experiment in this respect. The biochemistry of the dissimilatory nitrate-reduction and the physiological characteristics of *Pseudomonas aeruginosa* as well as its aerobic endogenous respiration have been widely studied. For this reason in the course of our investigations, we wanted to get data on this microbe as regards the characteristics of anaerobic endogenous nitrate reduction too.

EXPERIMENTAL RESULTS

Pseudomonas stutzeri: It can be seen from Fig. 1 that washed cells of *Ps. stutzeri* transform 3.5 micromoles/ml nitrate without adding substrate as electron donor. It is characteristic for the denitrification processes that firstly all of the nitrates turn into nitrite and the reduction of nitrite starts only when there is no nitrate already in the environment. The speed of the process is higher in the nitrate-reduction phase than in subsequent nitrite reduction phase. Repeated investigations pointed out that the dynamic characteristics for this process are similar but the speed depends on the conditions of precultivation.

The curves characteristic for endogenous nitrate-reduction are indicated in Fig. 1 and concern those cases when the amount of nitrate is shown in zero time 7.0 and 10.5 micromoles/ml. In the first 50 minute period as a result of increasing the amount of nitrate, the speed of nitrite formation is the same as in the case of the 3.5 micromoles/ml. After that, though more slowly, the amount of the produced nitrite increases but the reduction processes do not start under the experimental period.

The curves in Fig. 2 demonstrate the nitrate-reduction processes on the effect of the successive nitrate loading. In this case, at the beginning of the experiment, 3.5 micromoles/ml nitrate was added to 9 parallel samples. At the stage of nitrite reduction, after 320 minutes (when 27—37 micromoles/ml nitrite-nitrogen was in the sample), another 3.5 micromoles/ml nitrate was added to a new series of samples (with 3 parallels). In addition besides nitrate, glutamic acid was added to a further new series of 3 parallel samples.

Fig. 1. Endogenous respiration of *Ps. stutzeri* during denitrification (2 mg cells/ml)

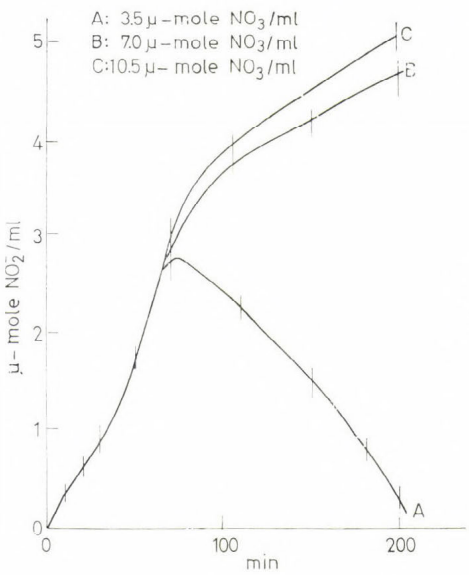
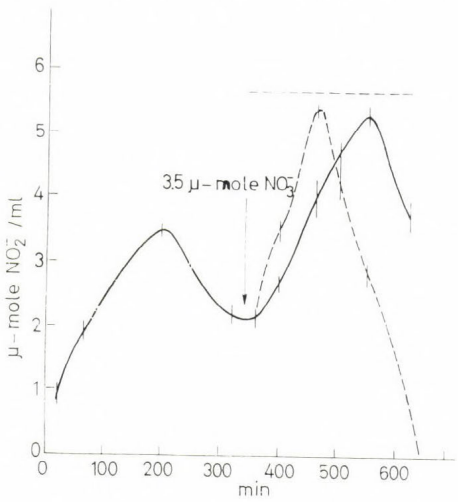


Fig. 2. Endogenous respiration of *Ps. stutzeri* under repeated loading with nitrate (1.7 mg cells/ml)



It can be seen from Fig. 2, that the cells carrying out nitrite reduction — after a short switch-over period — started to utilize the nitrate again as a result of the new supply of nitrate independently of whether nitrite is present in the environment or not. This has been confirmed unequivocally by the fact that the nitrite remained back from the first stage and the nitrate supplied in the second stage can be determined in the 550th minute of the experiment in the form of nitrite. After this time the amount of nitrite decreased.

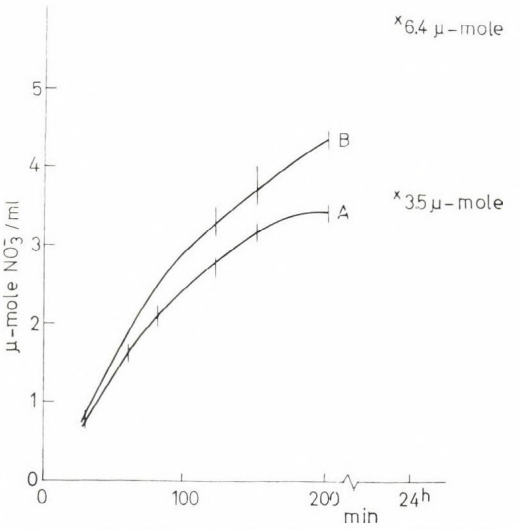


Fig. 3. Endogenous respiration of *B. licheniformis* with NO_3^-

It is likely that the reserve nutrients accumulated in the cells during precultivation were not sufficient for the utilization of 7.0 micromoles/ml material. It has been proved by the data obtained with the sample treated with glutamic acid where the supplied nutrient permitted a perfect utilization of a higher dose of nitrate. In this case too, it was experienced that *Ps. stutzeri* primarily utilizes nitrate and only after that starts to utilize the nitrite.

Bacillus licheniformis. As is obvious from Fig. 3 the endogenous respiration of *Bac. licheniformis* is similar to that of *Ps. stutzeri*, in the presence of nitrate under anaerobic conditions. As a result of this, the 3.5 micromoles/ml nitrate can be detected quantitatively as nitrite at the end of the experimental period. However the reduction of nitrite is not detectable during endogenous respiration. In other experiments the consumption of the produced nitrite was measurable too in the presence of some electron donors, but if the cells were cultivated on these, centrifuged and washed, and the endogenous respiration was examined the nitrite reduction was also not demonstrable.

The nitrite produced in the course of endogenous respiration in the presence of 7.0 micromoles/ml nitrate is shown on the "b" curve of Fig. 3. The speed of the nitrite formation was nearly the same as in the case of 3.5 micromoles/ml up to 220 minutes of the experimental period.

Pseudomonas aeruginosa. Preliminary investigations indicated that the *Ps. aeruginosa* utilizes the nitrate during endogenous respiration but nitrite is not produced and does not accumulate during the process.

Endogenous respiration in the presence of nitrite.

Pseudomonas stutzeri: It can be seen from Fig. 4 that *Ps. stutzeri* proceeds in endogenous respiration in the presence of nitrite as an initial material too. 3.5 micromoles/ml nitrite as initial material is consumed from the medium. In the case of 7.0 micromoles/ml nitrite, the nitrite reduction

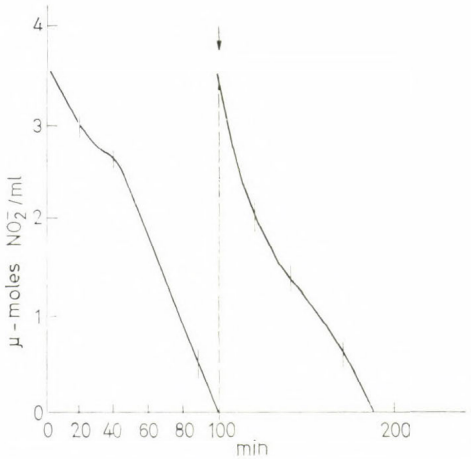


Fig. 4. Endogenous respiration of *Ps. stutzeri* under repeated loading with nitrite (1.7 mg cells/ml)

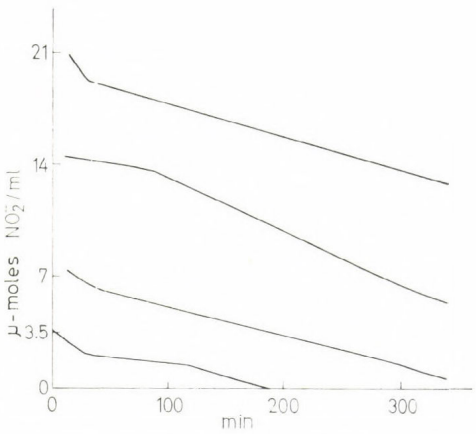


Fig. 5. Endogenous respiration of *Ps. stutzeri* during denitrification (2.8 mg cells/ml)

was not experienced. If 7.0 micromoles/ml nitrite is added periodically to the cells — at zero time 3.5 micromoles/ml and later the same amount again — the whole amount of nitrite will be utilized. This indicates that 7.0 micromoles/ml inhibits the life functions of cells.

Nitrite reduction of Ps. aeruginosa in the course of endogenous respiration was investigated in the presence of 3.5, 7.0, 14.0, 21.0, micromoles/ml nitrite. The results are presented in Fig. 5. It can be seen that there was no significant inhibition in the case of 21 micromoles/ml concentration. The speed of the nitrite reduction was the same in all nitrite concentrations applied. Nitrite reduction of *Bac. licheniformis* was not detectable during endogenous respiration.

SUMMARY

1. *Ps. stutzeri*, *Ps. aeruginosa*, and *Bac. licheniformis* utilize nitrate as electron acceptor during the anaerobic endogenous respiration.

2. a) In the course of anaerobic respiration of *Ps. aeruginosa* nitrite accumulation could not be detected.

b) In the case of *Ps. stutzeri*, the whole quantity of nitrate entirely transforms into nitrite and in the next stage the accumulated nitrite is utilized.

c) The characteristic feature of anaerobic endogenous respiration of *Bac. licheniformis* is that nitrite develops from nitrate and accumulates in the environment.

3. Nitrite as electron acceptor can be used by the two pseudomonads but not by *Bac. licheniformis*.

4. As a consequence of an increase in the nitrate concentration in the case of *Ps. stutzeri* the formation of nitrite was increased but the nitrite

produced is only partially utilized. In the case of increased doses of nitrate the speed of nitrate reduction is approximately the same. 7 micromoles NO_3^-/ml also entirely transforms into nitrite.

5. It appears from our experiments carried out with increasing quantities of nitrite that 7 micromoles/ml NO_2^- inhibits the life functions of the cells of *Ps. stutzeri*. The same quantity of nitrite, if periodically supplied is completely reduced.

6. In the case of *Ps. aeruginosa* a lower degree of inhibition is experienced only in the presence of 21 micromoles NO_2^-/ml. 3.5, 7, 14, 21 micromoles/ml decreases in the medium and is subsequently utilized.

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UTILIZATION OF AMINO ACIDS BY *PSEUDOMONAS STUTZERI* UNDER AEROBIC CONDITIONS AND DURING NITRATE RESPIRATION

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Investigating the carbohydrate catabolism *Pseudomonas stutzeri* during aerobic and nitrate respiration, Spangler and Gilmour found that there was no major shift in the patterns of glycolysis and pentose-phosphate cycle, but the amount of CO_2 originating from the C—2, C—3, C—5, C—6 atoms of glucose or gluconate increases during the nitrate respiration to the disadvantage of the body-building process. Their observations are in agreement with Elliot's (1965) work on oxidative phosphorylation in *Ps. stutzeri* which indicated that under aerobic conditions, 3 molecules of ATP may be formed for each oxygen atom consumed and 2 molecules of ATP per NO_3^- ion consumed under conditions of nitrate respiration. Considering this evidence to be applicable, one would expect that a greater recycling of the TCA cycle would be necessary for an equal amount of energy under conditions of nitrate respiration. The radio-respirometric investigations cited above really demonstrated the increased intensity of terminal oxidation.

Besides carbohydrates, some amino acids are the main sources of the TCA cycle. For this reason, it could be supposed that there were some differences in the metabolism of amino acids during aerobic respiration or nitrate respiration. On the basis of these hypotheses the following investigations were done.

MATERIAL AND METHODS

Test organism and media.

Pseudomonas stutzeri NNRL B—927 (CCEB—522) was used as a test organism in this study. The strain was maintained on potato infusion medium which has the following composition; potato infusion 125 g; NaCl 5.5 g; peptone 10.0 g; glycerol 10.0 ml; agar-agar 15 g; distilled water to 1000 ml. For amino acid analysis the following medium was used; 0.15 M PO_4^--buffer, pH 7.4, 1000 ml. Casamino Acids, Vitamin Free (DIFCO) 0.350% trace element solution (Pfennig 1961) 0.01%. In the case of anaerobic treatments the medium contained 0.2 g/l KNO_3 too.

The media were autoclaved at 15 psi for 10 min. The incubation was carried out in Erlenmeyer flasks which contained 50 ml of media and were aerated in the anaerobic treatments, or gassed with N_2 (O_2 content below 30 ppm) in the anaerobic ones. The sterilization of gases was conducted by bubbling them through a diluted chromic acid solution. The speed of flow checked with rotameter, was 20 ± 1 l/h in both cases.

Amino acid analysis

After six days' incubation, the cultures were centrifuged and 5.0 ml samples of the supernatant were concentrated, then heated to dryness on a water bath. The residue was extracted with 5.0 ml of the following buffer: Na-citrate 19.60 g; HCl cc 16.50 ml; thiodiglycol 20 ml, Brij 35 (20%) 4.50 ml; octanoic acid 0.1 ml, distilled water (boiled) to 1000 ml. The slime produced by the *Ps. stutzeri* was not soluble under these conditions and it was removed by filtration. Aliquots (0.6 ml each) of the water-clear extracts were applied to the columns of a BioChrom Amino Acid Analyzer which qualitatively and quantitatively analyzed the amino acid content of the samples according to the ,,BioRad Accelerated Low Pressure System.''

Determination of growth yields

The cell mass produced in each treatment was measured by means of a Pye Unicam SP 800 B spectrophotometer at 660 nm. A calibration curve was done for this measurement, plotting dry matter content against extinction of concentrated cell suspension. This was linear from 0.1 to 1.25 mg dwt/ml.

Chemicals

The chemicals used in this work were of the best quality products available commercially. Chemicals required for the amino acid analysis were supplied by BioRad Laboratories; the components of media were Difco's products.

RESULTS AND CONCLUSIONS

Data of measurements in a summarized form can be found in Table 1 from which a few more important correlations are easily visible. It is most essential that the growth yield of *Ps. stutzeri* decreased by about 10% under aerobic conditions on the effect of nitrate, and by carrying out denitrification the decrease was more than 80%. At the same time, on a dry weight basis, the utilization of amino acids increased by 25% and 200% respectively. The cation surplus, equivalent to the utilized nitrate increases the pH and as a consequence of this, the E_H decreases.

As it was expected, there were changes in the spectra of utilization on the effect of nitrate. In these deviations a few more or less complicated rules could be recognized which will be discussed in order of the different treatments.

Aerobic respiration

The evaluation of data of Table 1 will be facilitated if the % participation of certain amino acids in the given mixture and in the whole utilization are considered. The measured values calculated in this way give the data

Table 1

Utilization of amino acids by Ps. stutzeri under different experimental conditions

Amino acids	Amino acid added, micromoles/ml	Amino acid utilized, micromoles/mg dwt.		
		Aerobic	Aerobic + NO_3^-	Anaerobic
Asp	1.34	1.69	1.86	3.99
Thre	0.85	0.92	1.18	5.40
Ser	1.11	1.06	1.55	5.06
Glu	2.00	2.48	2.80	11.50
Pro	0.65	0.70	0.98	4.08
Gly	0.79	0.99	1.11	2.85
Ala	1.09	1.36	1.54	4.32
CySH	0.0059	0.0072	0.0084	0
Val	1.03	1.21	1.46	0
Met	0.42	0.45	0.59	2.30
i-Leu	0.79	3.20	3.70	0
Leu	1.87			0
Tyr	0.28	0.25	0.39	1.35
Phe	0.22	0.19	0.28	1.00
Lys	1.38	0.69	1.22	2.02
Hys	0.34	0.25	0.47	2.35
Arg	0.45	0.19	0.44	1.69
NH_4^+	1.26	1.15	1.18	7.05
Uptake (without NH_4^+), micromoles	2.66	15.63	19.61	47.92
Uptake (without NH_4^+), μg		2049.5	2561.3	6082.0
Growth yield mg/ml		0.800	0.710	0.145
pH at end of incubation		8.06	8.92	9.32
E_H at end of incubation, millivolts		+288	+276	+212

of Table 2. It should be noted that the data concern the actual amounts of amino acids because the applied casein hydrolyzate contained — according to the producers communication — about 40% inorganic salts. (According to our measurements the main components of this fraction are the following; Cl^- 22.2%, Na^+ 15.15%, K^+ 1.26%, Ca^{2+} 0.14%.)

It could be established that under aerobic conditions, *Ps. stutzeri* prefers some amino acids from the point of view of utilization. Keeping in mind the total amount and the utilization shift it was noticeable that there was a greater preference for glutamate, aspartate, leucins, alanine and glycine. In contrast with this, to a smaller extent serine, proline, tyrosine, phenylalanine and to a greater extent the more or less basic hystidine, arginine, lysine become more significant as regards utilization. Especially the uptake of lysin is very small, if one considers that there is more from it in the given mixture than from aspartate, but its utilization does not reach that of 50% of aspartate.

The total amino acid utilization on dry weight basis was 2.049 mg so the "incorporation" was 49.8%. This data agreed with the findings of Spangler and Gilmour mentioned in the introduction.

Table 2

Utilization of amino acids by Ps. stutzeri under aerobic conditions

Amino acid	Added, per cent		Utilized, per cent	Difference
Asp	8.45		10.08	1.63
Thre	5.35		5.48	0.13
Ser	7.00		6.31	−0.69
Glu	12.61		14.80	2.19
Pro	4.09		3.69	−0.40
Gly	4.97		5.90	0.93
Ala	6.86		8.10	1.24
CySH	0.03		0.04	0.01
Val	6.50		6.70	0.20
Met	2.64		2.68	0.04
i-Leu	4.97	16.77	19.05	2.28
Leu	11.80			
Tyr	1.76		1.49	−0.27
Phe	1.38		1.13	−0.25
Lys	8.70		4.11	−4.59
Hys	2.14		1.49	−0.65
Arg	2.85		1.13	−1.71
NH_4^+	7.90		6.85	1.05
Altogether	99.99		99.03	

Aerobic respiration in the presence of nitrate

This treatment needs some explanation because the nitrate respiration is an anaerobic process. But according to the results obtained so far (Lenhoff et al. 1956) changing from aerobic respiration to nitrate respiration does not occur at a defined oxygen tension but it happens with a continuous transition. For this reason, it can be supposed, that if the nitrate respiration causes changes at substrate level these should be still detectable in the metabolism of the respiring cells which are primarily aerobic. The measured data prove this theory. First of all, the small decrease in the growth yield was perceptible beside the increased amino acid transport. The "incorporation" decreases to 40.0%. There is an essential change in the utilization spectra of the amino acids. The majority of the amino acids were taken up according to their ratios in the given mixture. The utilization differences noticed in aerobic conditions were rather faint. Only the relatively low level of uptake of phenylalanine, arginine, lysine remained characteristic.

Nitrate respiration

The growth yield of the investigated strain decreased to a great extent, but at the same time the utilization of amino acids considerably increased. The amino acid utilization was 6.082 mg/mg dwt (Table 1), the incorporation calculated from this was only 17.3%. This was considerably less than that observed by Spangler et al. (1966) during their investigation on carbohydrate catabolism of *Ps. stutzeri*. Inspite of this, our data do not absolutely

contradict theirs because the author obtained the seresults with an other strain and in substantially different experimental conditions. In the first place, the important difference in the time of incubation (a few hours against six days) could cause this deviation. Taking into consideration the utilization of certain amino acids it can be established that their uptake is not in correlation either with their concentration or their simple physico-chemical properties (Table 2). Calculating anaerobic/aerobic quotients it could be observed that the utilization of asparagine, glycine, alanine, lysine were suppressed to some extent but the uptake of hystidine and arginine increased above the average level. These changes could be expressed with the concentration ratios and do not seem to be very important. In contrast to this, it is necessary to note that the participation of glutamic acid in the total utilization was 27.5%, which is obvious compared to its given quantity. This is in accordance with the glutamate-preference observed during the cultivation of this strain.

Four amino acids namely valine, leucine, i-leucine and cysteine were not utilized by the strain under conditions of nitrate respiration. The previous three of them can be measured back in unchanged form, and cysteine which was oxidized to cystine during processing of samples could be remeasured quantitatively too. The ceasing of the utilization of amino acids mentioned above could be connected with the colony structure changes of *Ps. stutzeri* which was already noticed formerly. Fresh isolates form wrinkled, dry, coherent colonies. Not long after the description of the species, still in the last century, it was Künemann (1898) who first reported the occurrence of mucoid variants. This dissociation was first connected by van Niel and Allen (1952) with the change in the aerobic mode of life or that carrying out denitrification. According to them, this characteristic colony-structure could be stabilized more or less by cultivating the strain on nitrate-containing media but the number of mucoid types would become larger with time. Stainer et al. (1966) found that one part of mucoid variants could regain its original structure if they were forced to carry out active denitrification for several generations. Our own experiments gave quite similar results, additionally it was detectable that the above-mentioned changes could be provoked by cold shock.

Therefore, on the effect of harmful environmental factors, *Ps. stutzeri* reacts by changes of cell wall synthesis and as a consequence of this with the change of colony-structure. The altered cell wall could cause differences in the utilization of certain amino acids, as it has been proved by Shockman et al. (1958) as well as other authors in connection with the sensitivity to antibiotics, UV irradiation, osmotic shock etc.

SUMMARY

Earlier investigations of other authors pointed out that in *Ps. stutzeri* the recyclisation of the TCA cycle increases during nitrate respiration while the incorporation from various ^{14}C-labelled atoms of glucose or gluconate considerably decreases. They suggested that this was the result of the energetically disadvantageous relations of nitrate respiration.

Because many amino acids have an important role in the metabolism of the TCA substrates, the author investigated the utilization of amino

acids by *Ps. stutzeri* NRRL B 927, (CCEB 522) under aerobic conditions and during nitrate respiration. The following results were obtained:

In the case of aerobic conditions the *Ps. stutzeri* utilizes all amino acids from the complex mixture of casein hydrolyzate. Glutamate, aspartate, the leucins, alanine were more preferable while the more or less basic amino acids namely hystidine, arginine, lysine less so. The yield of cellular material — on dry weight basis — was about 50% of amino acids utilized.

Under aerobic conditions, with added nitrate, the production of cellular material decreased. Each amino acid is utilized according to the ratio in the given mixture, except phenylalanine, arginine lysine which seemed to be less preferable.

During nitrate respiration four amino acids were not utilized by this strain. (Val, Leu, i-Leu, CySH). The utilization of the remaining highly increased to the disadvantage of body-building processes. The uptake of amino acids did not correlate with their concentrations or with simple physico-chemical properties.

These results are in agreement with results obtained by other authors from different aspects.

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REDUCING ABILITY OF WATERS AND SEDIMENTS; AN INDEX OF ENERGY CONSUMPTION RATE

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The first redox measurements were carried out in limnology by Karsinskin and Kuznetsov (1932), and ever since this first attempt redox potential came into the foreground of interest (Kuznetsov 1935, Persall and Mortimer 1939, Hutchinson et al. 1939, Deevey 1941, Allgeier et al. 1941).

The classical work of Mortimer (1941, 1942) about the relationships between the material circulations occurring at the mud-water interface and the redox condition once again directed the attention of researchers to the redox processes (ZoBell 1946; Hayes et al. 1958, Gorham 1958).

At this time, it has been pointed out by Hayes (1958) that in carrying out redox measurements unexpected difficulties may arise, quite recently Stumm (1967) passed severe criticism as to the practicability of directly measured redox potential in natural, mixed systems. Contrary to this, at the same time Berchardt (1967) and Wagner (1967) stated that the directly measured redox potential within a natural, mixed system yields valuable and practicable information.

Rabotnova (1957) gave a very detailed analysis on the properties of the redox potential of biological objects, and established that it differed in many respects from the properties of the conception used in chemistry. Whitfield (1969) considered the problems arising in connection with redox measurements from the point of view of a limnologist, and in order to describe the distribution of reduced and oxidized sediments he used the E_H as operational parameter with good efficiency.

Consequently it is reasonable and important to know and carry out measurements with regard to the momentary redox state of waters and sediments from the point of view of limnology. This is supported by the continuous increasing number of measurements carried out far and wide (Drabkova 1966, Romanenko 1966, Mikhaylenko 1967, Patrick and Turner 1968, Whitfield 1969).

The momentary redox state characterizing the different waters and sediments as a concrete ecological factor is a result of the complicated redox processes and with its measurements we can gain information about the energy consumption processes in the natural substrate very seldom. The redox state of the mud-water interface in a stratified lake with suitable trophic level for instance can give information about the energy consumption processes within the lake itself.

With the measurement of the momentary redox state in the Hungarian shallow and very extensive lakes we are not able to investigate the energy consumption processes and so we have elaborated a new method to gain some information on these processes.

MATERIAL AND METHODS

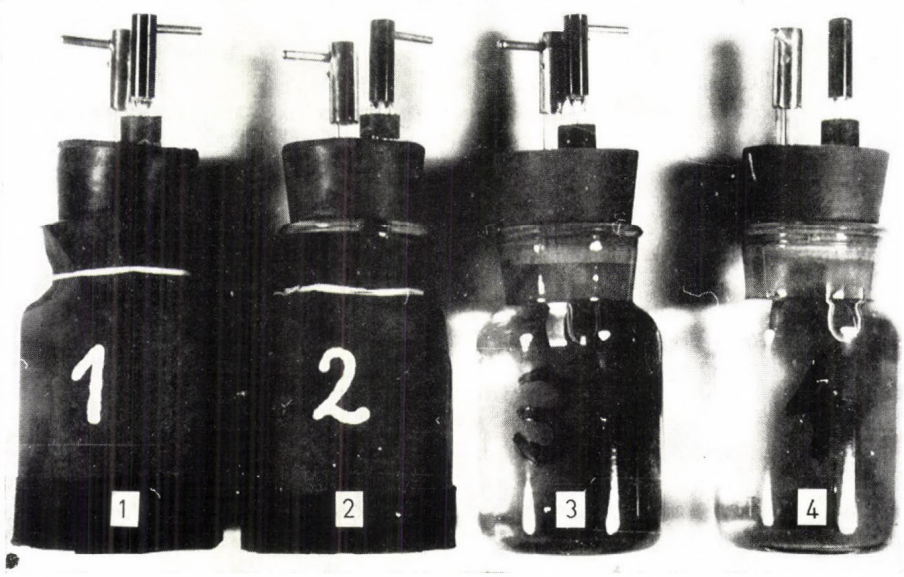
The measuring vessel (Fig. 1), was a 250 ml glass container of 6.5 cm diameter fitted with a glass or rubber stopper. The measuring and reference electrodes built into the stopper were immersed during the analysis into the liquid to be measured. By completely excluding oxygen diffusion it could not solve the problem of coupling the reference electrode into the measuring space with agar bridge or its simplified variety (Kovács and Matkovics, 1954).

We incubated our samples at 25 °C, in the case of the dark parallels the measuring vessel has been covered with aluminium sheet. The light parallel has been illuminated by 5000 Lux. The measuring vessel was filled excluding all bubbles and the samples were saturated with oxygen by bubbling through them air at 25 °C before filling up.

During our investigations we used electrodes manufactured by Radelkis (Electrochemical Instruments, Budapest). The measuring electrode is a smooth platinum sheet with a surface area of 2×0.5 mm. It has been washed before application in chromic acid, followed by a careful rinsing in distilled water. The measuring electrodes were calibrated by ZoBell's solution (1964). Our reference electrode was a saturated calomel electrode. The measurements were carried out in a Beckman GS-type pH meter and to the values thus obtained we added 250 mV and so the final values were given in E_h. Working with closed systems, the repeated measurements could be reproduced with an exactness of $\pm$ 10 mV.

Our investigations were carried out with waters and sediments from Lake Balaton and Lake Belső (lake of the peninsula Tihany). To determine

Fig. 1. Measuring vessels prepared for dark and light incubation



the reducing ability of the sediment we placed some 50 g moist mud into the measuring vessel, then at 25 °C oxygen-saturated lake water was layered on it.

By differential filtration using Soviet and Oxoid filters (pore size: 100 μ, 6 μ, 0.5 μ) we were able to measure separately the role of zoo-, phyto- and bacterioplankton in the reducing ability of the waters. The oxygen concentration during the investigation was determined by the Winkler method. The microbiological investigations were carried out as usual (Oláh 1970).

RESULTS

When the lake water was incubated in light the redox potential within the measuring vessel remained unchanged for a long period of time, or it increased (Fig. 2). Even during 42-day incubation no decrease in redox potential was observed. When the same samples were incubated in dark — i.e. excluding photosynthesis — after a period of time the redox potential decreased. This phenomenon may be called the darkness induced reducing ability of the sample. The main characteristics of the curve obtained during incubation in dark: 1. The time of redox potential fall to the anaerobic equilibrium; 2. The duration of the decreasing section; 3. The redox potential at the anaerobic equilibrium.

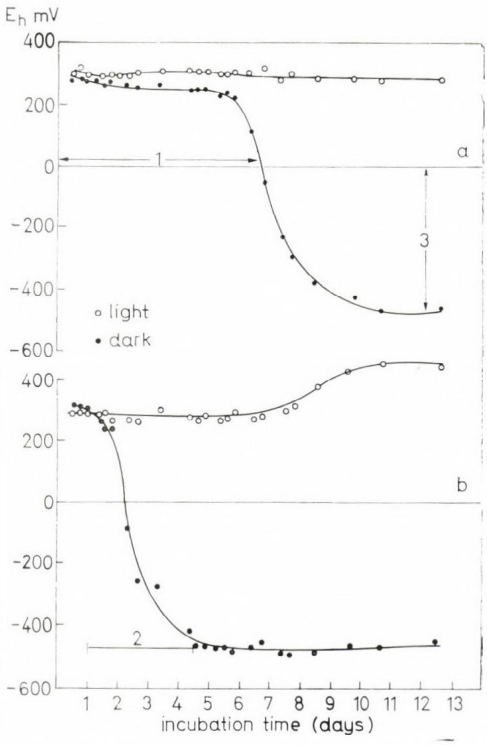


Fig. 2. Changes in redox potential during dark and light incubation, *a)* surface water; *b)* botton water from Lake Belső

Excluding the continuous oxygen supply in the measuring vessel gradually resulted in the total consumption of oxygen (Fig. 3) which in turn sets off further processes causing an even more pronounced decrease in redox potential. The length of time until the decrease in redox potential set in (1), consequently depends primarily on the intensity of oxygen consuming processes. The significant decrease in redox potential coincided with the complete disappearance of oxygen, thus, we obtained data as to the rate of oxygen consumption by observing the darkness-induced reducing ability and furthermore we may obtain information on the biochemical oxygen demand of natural waters and sediments. By knowing the oxygen content of an oxygen-saturated water at a given temperature and the volume of the measuring vessel we can calculate the quantity of the oxygen used during a given period of time.

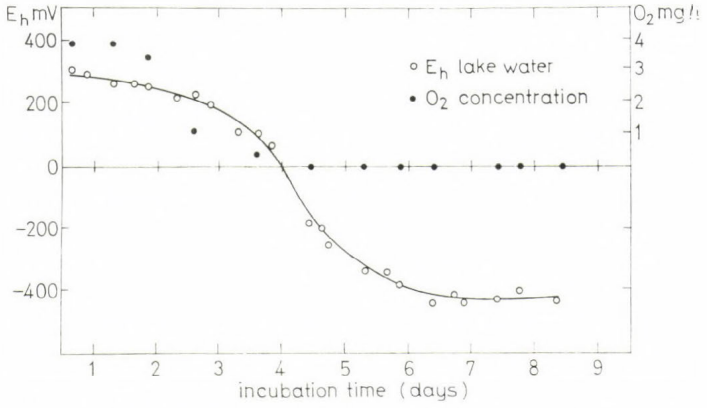
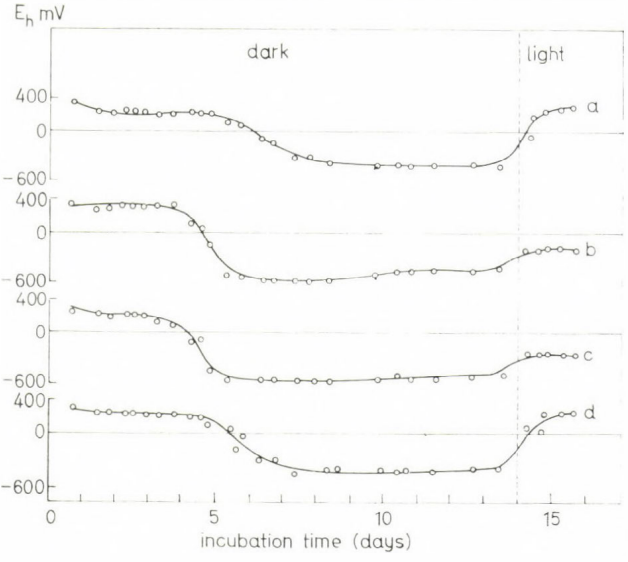
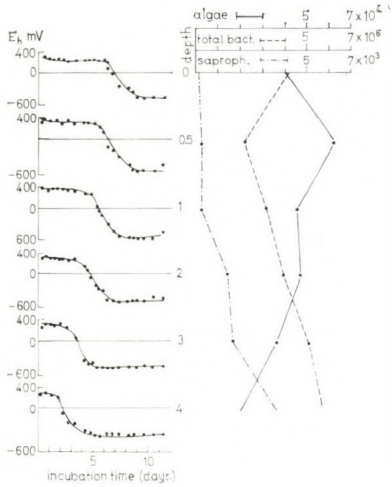


Fig. 3. Relationship between redox potential and oxygen concentration changes during incubation of Lake Belső water

The redox potential however, independently from oxygen concentration, suffered further changes under the influence of several other factors. From this fact it follows that by simply measuring the concentration of oxygen we do not get a picture true to reality as regards actual redox state. In the oxygen-free period in the state of equilibrium the redox potential (3) at any rate depends on the special biological and chemical composition of the sample. The length of the decreasing par t(2) on the curve, on the other

Fig. 4. Influence of glucose and dark, then of light incubation on the reducing ability of Lake Belső water, *a)* lake water + 2 mg/l glucose, *b)* lake water +20 mg/l glucose, *c)* lake water +40 mg/l glucose, *d)* lake water





F ig. 5. Relationship between the redu cing ability of various water layers a nd the vertical distribution of plankton in Lake Belső

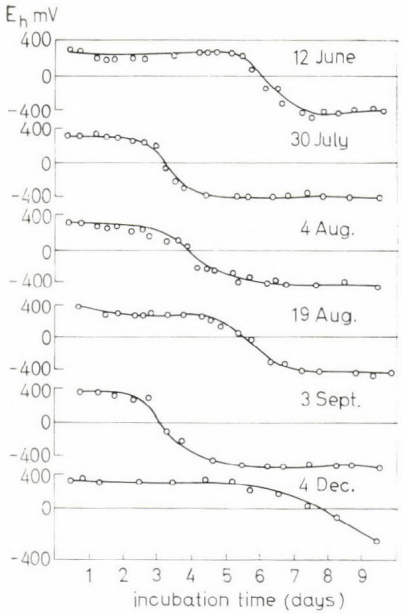


Fig. 6. Seasonal changes in the reducing ability of the Lake Belső water

hand, depends additionally on the change in oxygen concentration besides the chemical and biological composition of the sample.

If the sample gaining the equilibrial state characteristic for oxygen-free condition, is placed in light (Fig. 4) its redox potential attains a value of the corresponding initial state. In the case of samples filtered through 6 μ sieve — without phytoplankton — reoxidation, naturally, cannot be affected. It is interesting to observe that after adding a 20 and 40 mg/l glucose regardless a slight increase in redox potential, the sample is not reoxidized. On the other hand, using a low concentration of glucose solution the process of reoxidation passes freely.

The complex vertical investigation carried out in Lake Belső (Fig. 5) shows the close relationship between the reducing ability and the living organisms. Redox potential decrease was measured in the bottom water on the 3rd day, while in the surface water on the 8th day. The time required for the decrease in redox potential from the surface water proceeding downward is gradually shortening by 1 day per meter. Parallel with this, the total quantity of microbial plankton gradually increases towards the bottom from 2×10^6/ml to 6×10^6/ml. The number of saprophytic organisms increased with a similar tendency from 200/ml to 3800/ml. On the contrary, the quantity of phytoplankton decreased towards the bottom. In the surface water the reducing ability is well-high the same down to a depth of some 50 cm, it is probable that the sudden decrease in the total microbial plankton at a depth of 50 cm is compensated by the maximum of phytoplankton existing there.

The darkness-induced reducing ability of the water from the Lake Belső displayed marked changes according to seasons. The biggest was (4 days) on the 30th July and 3rd September, while the smallest was (9 days) on the 4th December (Fig. 6).

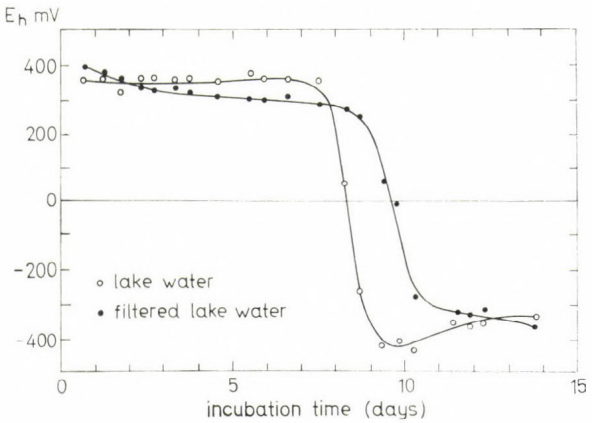
The vertical examination of Lake Belső proved that the reducing ability of waters and sediments was primarily determined by the quantity and quality of living organisms besides the nutritional supply. By differential filtration the effects of individual components could be well separated. Significant difference was observed between the reducing ability of the filtered and unfiltered water of Lake Belső and that of Lake Balaton.

The 6 μ filtration of the water of Lake Balaton (Fig. 7) shifted the decrease of the redox potential by a day and half, and the so obtained state of equilibrium hardly showed any difference from that of unfiltered water.

Examining the same in Lake Belső on the 30th July (Fig. 8), the 6 μ filtration shifted the decrease of the redox potential by three days, on the other hand, the so acquired state of equilibrium was by 300 mV more positive than that of the unfiltered water. The separation of zooplankton shifted the decrease of the redox potential only by one day and the so gained state of equilibrium hardly differed from the previous state. Consequently in the case of Lake Belső the phytoplankton plays an important role in the formation of redox potential corresponding to the oxygen-free equilibrial state.

The darkness-induced reducing ability of natural waters and sediments may be used in studying the processes of mineralization and by their help we were able to measure the influence of various materials exerted on redox potential. Under aerobic condition the mineralization of organic materials was proportional with oxygen consumption. From the quantity of consumed oxygen, and from the rate of consumption we may conclude the intensity of mineralization. On the other hand, under anaerobic condition the mineralization products exerting an effect on the redox potential was dominant.

Fig. 7. Reducing ability of the filtered and unfiltered water of Lake Balaton



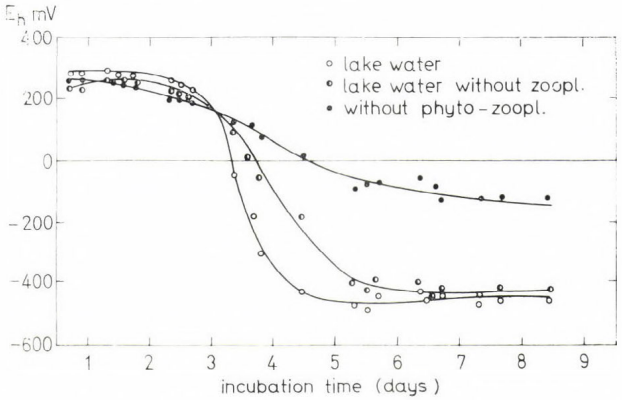
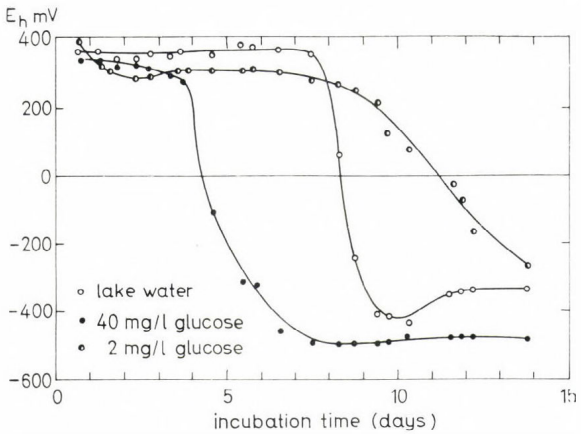


Fig. 8. Reducing ability of the filtered and unfiltered water of Lake Belső

On giving to the water of Lake Balaton 40 mg/l glucose, (Fig. 9) we found that the length of time required for the decrease in redox potential was 4 days shorter. Using a 250 ml measuring flask at 25 °C with a 100% saturation the consumption time of 2 mg oxygen decreased to its half. We carried out the same experiment with the water of Lake Belső, whose time requirement for the decrease in redox potential is shorter than that of Lake Balaton. Under similar conditions with a 40 mg/l glucose the length of time required for a decrease in redox potential is only one day shorter. Therefore by adding glucose the required time for a decrease in redox potential for the water of Lake Balaton and Lake Belső becomes balanced. On the effect of glucose the redox potential corresponding to the equilibrial state both in the cases of Lake Balaton and in the Lake Belső decreases by nearly 100 mV. On the 19th August, 1969 in the case of water of Lake Belső free of phyto- and zooplankton the time required for a decrease in redox

Fig. 9. Influence of glucose on the reducing ability of Lake Balaton water



potential lengthened by 2 days compared to the unfiltered water. Comparing it to the one measured on the 30th July the redox potential corresponding to the state of equilibrium it became more positive only by 50 mV. This clearly indicates that even within a season in a lake the role of individual components change in the formation of the reducing ability. When we added to the filtered lake water of Lake Belső a 40 mg/l glucose we obtained a result very similar to that of the unfiltered water. Consequently at the time of examination the role of bacteria was decisive in the process. It was interesting to note, that giving to the water of Lake Balaton and to the Lake Belső a 2 mg/l glucose caused a 4 and 1 day shift in the decrease of redox potential. When the same concentration was given to the water filtered through a 6 μ filter deriving from Lake Belső the length of time required to cause a decrease in redox potential doubled. Comparing the redox potential corresponding to the state of equilibrium to the control a 200 mV higher potential was measured while the same compared to water to which a 40 mg/l glucose was added this value reached 400 mV.

So far we have no explanation to the phenomena accompanying the addition of glucose with low concentration.

DISCUSSION

The intracellular rH_2 value depends on the redox state of many systems activated by ferments and is unstable. It is about 20 under aerobic conditions and decreases rapidly when the environment becomes anaerobic. In the case of microorganisms the relationship between the state of intracellular redox systems and the external environment is especially close. The hydrogen acceptor for the oxidation of the substrate is the environmental oxygen. If the oxygen supply is limited a whole series of redox systems is reduced by hydrogen and the reduced substances are accumulated inside and outside of the cell decreasing the redox potential in the environment (Rabotnova 1957).

On the basis of these it was easy to understand that measuring the reducing ability of the mixed natural systems and their components we can get informations about the total biological activity, more exactly about the mineralization of organic substances or the utilization of energy included in other reduced substances (H_2S, NH_4^+, Fe^{++}).

With the measurement of the reducing ability of waters and sediments we can investigate the rate of the total energy utilization. Besides the energy-producing processes this is the most important possibility for the estimation of trophic level especially in lakes with allochthon and bifocal organic flux under which condition to measure the rate of the energy production is very difficult.

Over the estimation of the trophic level the reducing ability has a concrete ecological significance. From its rate the possible momentary redox state of natural substrate can be calculated and this is one of the most important integrative properties of waters (Dussart 1967).

From the hypolimnetic oxygen deficit in stratified lakes Strom (1931) calculated the productivity and at the same time called the attention to the limits of the method. In oligotrophic lakes the oxygen deficit is too

small to classify the lake, on the other hand in highly eutrophic lakes where in winter the oxygen is completely consumed in the hypolimnion it is impossible to estimate the total reducing ability of sediment.

Mortimer's method (1941, 1942) to estimate the trophic level using the winter thickness of the oxidized microzone is based also on Strom's idea. Mortimer's method was criticized by Gorham (1958).

Strom's idea was utilized in Hayes and MacAulay's work (1959). To indicate the trophic level they used the oxygen consumption rate measured under laboratory condition. Their results show the advantages of the laboratory measurement in comparison with Strom's in vivo method.

The oxygen consumed during the mineralization of organic substances has been determined to assess the intensity of organic matter turnover by the Russian aquatic microbiologists, too (Kuznetsov and Romanenko 1968).

The largeness of the oxygen consumption does not inform about the energy consuming processes under anaerobic condition and so our method for the measurement of the total energy consumption rate is more suitable. The measurement of the reducing ability for the study of the rate of energy consumption is especially valuable in our shallow, extensive lakes, where due to the constant atmospheric oxygen supply even at the mud-water interface we are not able to get any information about this process with the measurement of the momentary redox potential.

SUMMARY

A simple method was applied for the measurement of the reducing ability of the natural waters and sediments. The method is based on the redox potential changes in a closed system containing the natural substrate.

The simple cell for the measurement is a glass vessel of 250 ml with platinum and calomel electrodes embedded in the rubber or glass stopper. The redox potential fall in the system is induced by the exclusion of the photosynthesis during the dark incubation and the oxygen supply from the air. After reilluminating the sample previously settled at the state of the anaerobic redox potential equilibrium the redox potential returns to the original value.

The obtained redox potential curve gives information on the following: *a)* The time of the redox potential fall to the anaerobic equilibrium; *b)* The duration of the decreasing section; *c)* The redox potential in anaerobic equilibrium.

The darkness-induced reducing ability is suitable for the study of energy-consuming processes taking place in our shallow, extensive lakes.

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INVESTIGATIONS ON ANAEROBIC PROCESSES IN THE FORMATION OF SOLONCHAK AND SOLONETZ SOILS

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In the first part of August 1955 a large committee studied the root disease of rice which appeared at that time in the Hungarian paddy fields. The damage in that year amounted to about a thousand million forints, and was limited to acidic meadow and solonetz soils formed on alluvial deposits of the rivers Tisza and Körös. Root rot did not occur in the valley of the Danube, on the solonchak soils. Figure 1 presents general information on the localisation of alkali soils in Hungary according to Stefanovits and Szücs (1961). Since there was no difference in the weather of the two areas, the cause of the damage could be attributed to the differences in soil properties.

The soil of the Hungarian rice fields is covered with water during the whole of the vegetation period, and in water-covered soils reduction processes take place at different intensities and H_2S is found among the reduction products. Certain plants, including rice, can resist the toxic products formed in the root zone by means of the oxidizing power of the roots. This oxidizing power is connected with the intensity of photosynthesis, or more correctly, with the undisturbed life processes of the plant (Vámos 1959, Armstrong 1969).

Investigating the causes of mass decay of fish, it was found that fish decay caused by H_2S is mainly limited to ponds and backwaters on old deposits of the Tisza and Körös. In the areas along the Danube, fish decay caused by H_2S is not known to occur.

We made investigations to find out why the damage caused by H_2S occurs only on heavy, acidic soils. It was found that in waterlogged soils, there were differences in the decomposition of organic matter depending on the mineral composition of the soil; the decomposition products in addition to the direct physiological effects play a part in the soil-forming processes. Thus investigation of the direct cause of the damage became identical with elucidation of the soil-forming processes.

MATERIAL AND METHODS

Besides meadow soils various solonchak and solonetz type soils were used in model experiments. Cotton was put between the layers of these soils in order to ensure by its fermentation the energy necessary for the reduction processes. Since we wanted to assist first of all the formation of H_2S, we ensured the nitrogen supply by adding $(NH_4)_2SO_4$. The soils

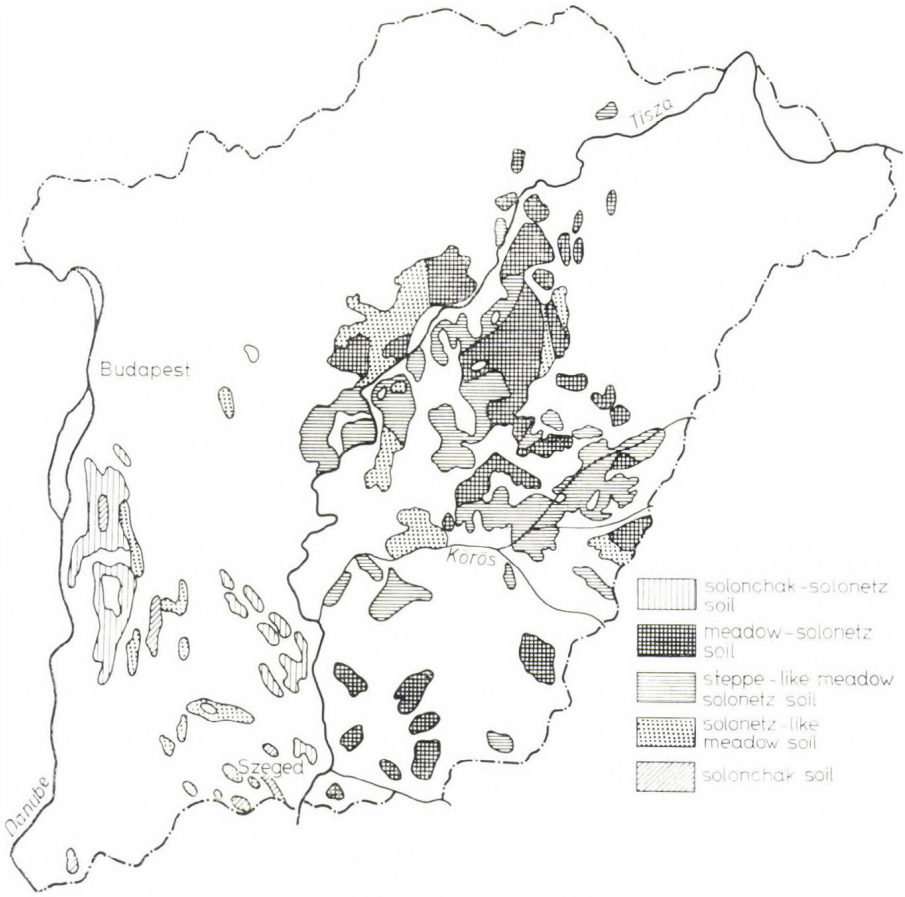


Fig. 1. Alkali soils on the Hungarian Plain

were provided with a 2 cm cover of tap water; then the dishes were put in thermostats at 25, 30 and 35 °C. (Fig. 2).

The presence or absence of sulphate-reducing bacteria, and their amount, was determined by the simple method described below.

One ml of the water to be examined was poured into a sterile test tube and 12 ml of Starkey agar solution was added. After coagulation of the agar 4—5 ml of bouillon agar was put over it in layers and this was later inoculated with *Serratia marcescens*. Serratia, as oxygen consumer, forms anaerobic conditions and provides the low redox potential necessary for the reduction. Blackening of the Starkey agar indicated the presence of sulfate-reducing bacteria. (Fig. 3).

RESULTS

1. In the experiments with heavy, acidic meadow and solonetz soils, the soil on both sides of the cotton, and to a degree the cotton itself, became black in three weeks due to ferrous sulphide. The amount of sulphide was determined calculating for 100 g of soil. (Fig. 4).

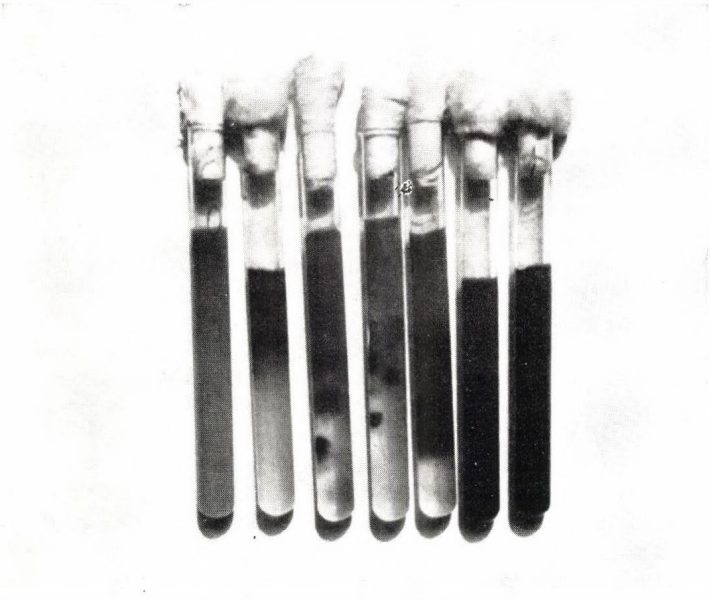


Fig. 2. Method for the determination of sulphate-reducing bacteria

Fig. 3. Method for determination of sulphide formed by sulphate reducing bacteria in model experiment

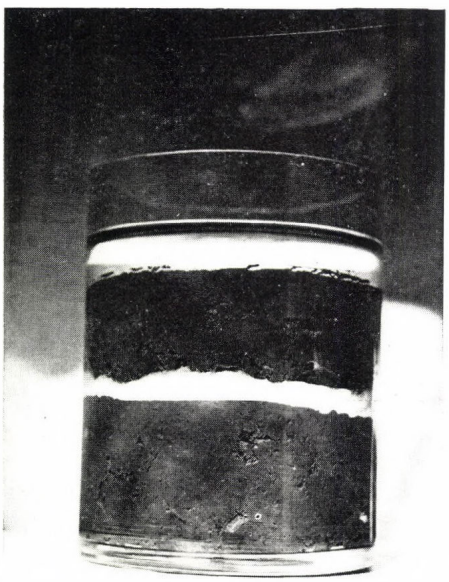
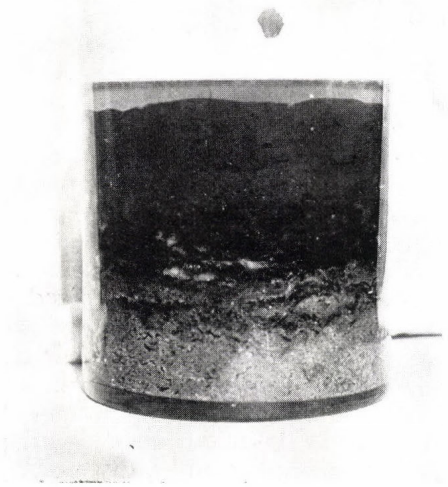


Fig. 4. Ferrous sulphide formation in water-covered acidic meadow soil



2. In the experiments with solonchak soils no ferrous sulphide was formed; however, the cotton did turn yellow. The yellow discolouration was caused by a pigment produced mainly by *Clostridium felsineum*.

3. In the next experiments we spread acid meadow soil under the cotton layer and over it solonchak soil from the valley of the Danube. In three weeks at 30 °C the upper surface of the cotton became orange-yellow, while the lower surface and the soil became as black as coal.

4. In the experiments with solonchak-solonetz soil from Fehértó near Szeged, no ferrous sulphide was formed. At 25 °C the cotton was completely decomposed in about 7 weeks with intense gas formation.

It appears from these results that the unfavourable effect caused by H_2S in ponds and rice fields can only be expected to occur on heavy acidic soils formed in former flooding areas of the Tisza and its tributaries.

The intensity of the reduction processes and the rate of the decomposition of organic matter in the case of different water covers were examined in further investigations. For this purpose the total numbers of bacteria were compared in the cases of 10 cm, 2 cm and 0.5 cm water covers. It appeared from these tests that bacterial activity, indicated by the number of bacteria, was greatest in the surface layer of waterlogged soils or in the case of shallow (5 mm) water cover. The oxygen consumption of the microorganisms multiplying on the surface of the soil or in very shallow water created an oxygen-deficient environment. (Table 1). The richer the

Table 1

Depth of water (cm)	Number of bacteria (million/ml)	
	Meadow soil	Solonchak-solonetz soil
0.5	136.2	52.2
2	69.4	58.7
10	24.0	17.0

surface layer of the soil in nitrogen, the quicker the anaerobic conditions form.

After the disappearance of nitrate, reduction of iron and manganese began in consequence of which the amounts of ferrous and manganous ions in the mud solution increased (Bloomfield 1959) (Figs 5, 6). The cause of this may be that the reducible iron and manganese oxides act as an acceptor of the electrons produced by bacterial respiration, and so the iron and the manganese may dissolve. Ponnamperuma (1964) mentioned that a large organic matter content of the soil, high Fe^{++} and Mn^{++} concentrations remained in the mud solution for a longer time than in a soil poor in organic matter. He also stated that after the drying-up of the pond-bottom, the oxidized iron colours the soil lilac-red. A toxic effect of the accumulating manganous ions can be expected in over-irrigated solonetz-solonchak soils, in acidic meadow soils, solonetz soils and in other soils rich in organic matter, where the amount of Mn^{++} ions in the soil solution remains for a long time around 40—50 mg/l. According to our experiments the manganous abundance has a significant role in the appearance of "damping off" disease of seedlings particularly in cloudy weather.

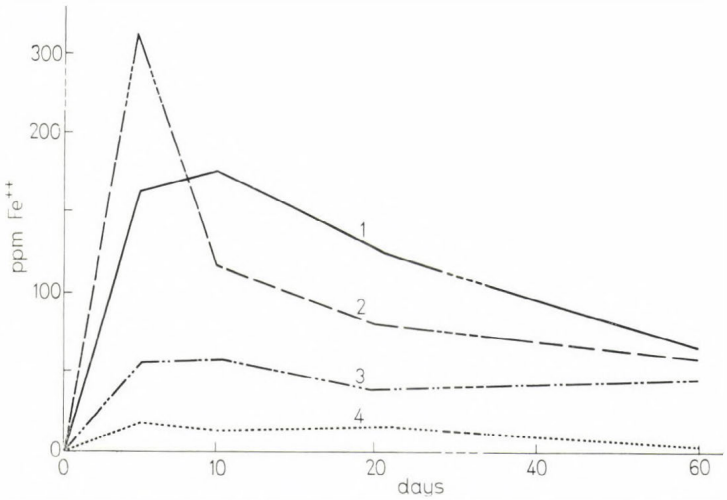
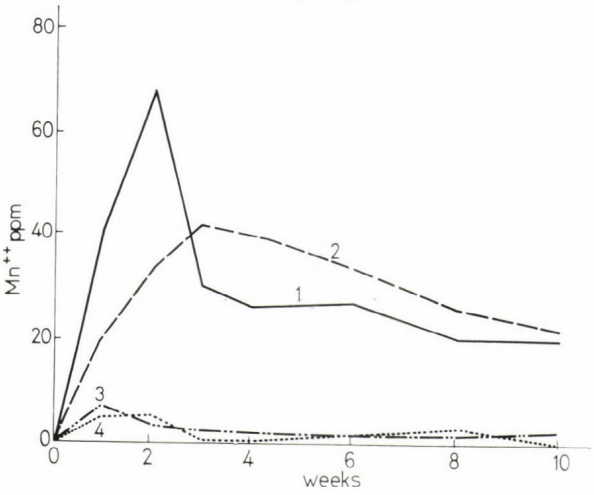


Fig. 5. Changes of Fe^{2+} concentration in submerged meadow-solonetz (1—2) and solonchak-solonetz (3—4) soils

In the case of a lower redox potential, sulphate reduction starts in consequence of which H_2S and $NaHCO_3$ are formed (Bloomfield 1969). Changes of SO_4^{--} and S^{--} amounts in submerged meadow-solonetz and solonchak-solonetz soils are shown in Fig. 7.

The H_2S reacts with the Fe^{++} ions formed in heavy, acidic soils and FeS accumulates (Bloomfield 1969). This does not, however, take place in the case of limy, sodic soils. There were much fewer Fe^{++} ions and much less ferrous sulphide formed. On cooling and decrease of the air

Fig. 6. Changes of Mn^{2+} concentration in submerged meadow-solonetz soils (1—2) and solonchak-solonetz soils (3—4)



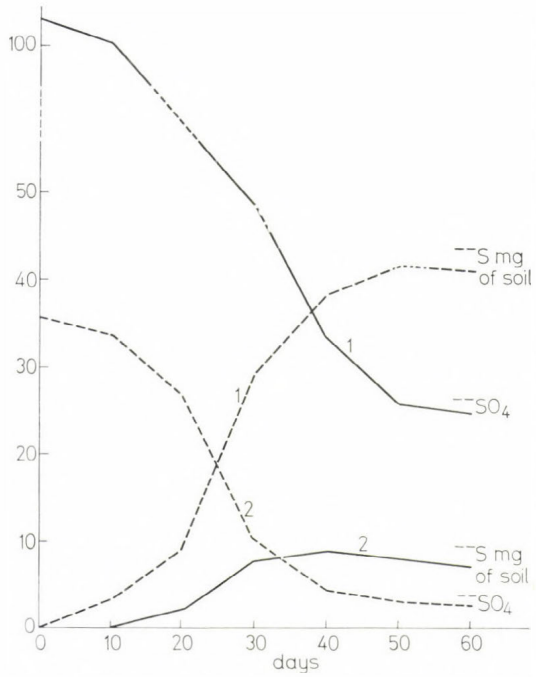


Fig. 7. Changes of SO_4^{--} and S^{--} amounts in 1) submerged meadow-solonetz and 2) solonchak-solonetz soils. S^{2-}: mg/100 g soil; SO_4^{2-}: mg/1000 ml water

pressure, hydrogen sulphide was liberated from the ferrous sulphide accumulated in the mud of acidic soils. The simplified equation for sulphide oxidation, consisting of several steps (Starkey 1966), may be written as follows:

$$2S^{--} + 3O_2 + 2H_2O = 2H_2SO_4$$

The released hydrogen sulphide causes the damage in the rice fields and fish ponds (Vámos 1964, 1968).

When the surface dries the high salt content hinders the decomposition of organic matter, and the pressure of CO_2 decreases. Sodium carbonate crystallizes on the drying surface but only where there is lime, for the lime neutralizes the sulphuric acid formed by the oxidation of sulphide and prevents the crystallized soda from changing back into Glauber's salt. However, the sulphuric acid plays a part in leaching the surface layer of the lime-free soil and in forming the solonetz soil profile.

With the help of test microorganisms we ascertained that the yellow pigment and other products of the Clostridia have no inhibiting effect on the common aerobic bacteria.

On the other hand, from vegetable matter decomposing under anaerobic conditions, compounds may form which inhibit the anaerobic processes.

Certain peats, such as some in North Europe, inhibit even the sulphate reduction itself. We found that such peat placed in Starkey's liquid culture medium hindered the sulphate reduction completely. Since H_2S could not be demonstrated even in traces, it is obvious that there is an inhibiting compound in peat. This compound, however, has no inhibiting effect on aerobic bacteria. If the peat is exposed to air and light, it quickly loses its antibiotic effect.

This finding of ours explains the preservation of human corpses found in North European peat bogs. When a corpse fell into the bog, it immediately sank under the redox level and so there was no activity of aerobic bacteria. In addition, the agent of the peat hindered anaerobic decomposition. For example, one corpse was preserved for thousands of years with a rope round the wrists and with undigested parts of the last meal in its stomach.

DISCUSSION

In 1839, Irinyi stated that the formation of soda is connected with the decomposition of organic matter because soda cannot be formed inorganically in nature. Muraközy (1902) was the first to recognize the importance of the biological processes taking place in stagnant waters in the formation of alkali soils. In connection with the investigation of the processes of alkali soil development, Treitz (1923) suggested the study of the cycle of organogenic elements as an important task, and he was the first to assert the possibility of soda formation through bacterial sulphate reduction. 'Sigmond (1922) stated that an arid climate, a periodic water cover and a water-impermeable layer in the soil are the factors of alkalisation. Without these factors, there would have been no development of anaerobic and aerobic conditions with redox processes resulting in an alkali soil profile. In the reducing process a weak acid (H_2S) and a strong base (NaOH) are produced. In oxidation, however, a strong acid (H_2SO_4) and a weak base (FeO—OH) are formed. According to the predominance of these processes alkaline (solonchak) and acidic (solonetz) types of alkali soils may develop. The most important factor in this development — as we have seen — is the presence or absence of lime.

In water-covered limy soil less ferrous iron and less FeS form. Some of the H_2S escapes into the atmosphere. In addition the lime protects the soda and thus the sulphuric acid formed under aerobic conditions cannot be reconverted to Na_2SO_4. Therefore during the course of time the amount of soda throughout the whole profile increases and crystallizes on the soil surface. This is the essence of the formation of solonchak soil.

On the other hand, in acidic soils much H_2S accumulates mostly in the form of FeS, and later, during desiccation, under aerobic conditions it is oxidized to sulphuric acid and transforms the carbonates into sulphates. The same process weathers the minerals of the environment and carries the dissolved salts into deeper layers. This is the essence of the development of solonetz type soils.

The root rot of rice plant indicates those sites where the formation of a solonetz profile occurs strongly. There is therefore no genetical relationship between the two types of alkali soils.

SUMMARY

Alkalization takes place in wet and dry periods and is a result of microbiological and abiotic transformation of organogenic elements, mostly of sulphur, nitrogen, and carbon. The preconditions of these transformations are the factors described by 'Sigmond: arid climate, periodical waterlogging and a water-impermeable layer. These factors involve the reduction and oxidation processes which are responsible for the forming of characteristic profiles of alkali soils depending on whether the swamp does or does not contain lime. From the former, solonchak and from the latter solonetz soils are formed. There is no genetical relationship between the former and the latter groups.

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SOME RESULTS OF THE FIELD EXPERIMENT "ETERNAL RYE CULTIVATION" IN HALLE (GDR) AFTER 90 YEARS' EXPERIMENTATION

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The single crop farming and fertilizing experiment "Eternal rye cultivation" in Halle is one of the oldest and best-known experiments in the world. It was commenced in 1878 by Julius Kühn, founder of the agricultural sciences at the University in Halle and was continued from this date in its original lay-out and intended purpose unchanged in principle.

With regard to the intended purpose, Kühn (1901) wrote that by uniform treatment over a long period insight should be gained with regard to the efficiency of the different fertilizers on soil and plant. In this connection it is of special interest to compare the results obtained with farmyard manure with that of an exclusive application of mineral fertilizers; furthermore, to determine the consequences of the omission of the supply of nutritive substances.

The results of this experiment, obtained hitherto, are available in a series of publications. Special mention should be made of those by Kühn (1901), Roemer and Ihle (1925), Scheffer (1931a, 1931b), Schmalfuss (1950, 1961), Merker (1956), as well as Kolbe and Stumpe (1969), who were responsible for the experiment at times.

The experiment is conducted with winter rye as test plant on a diluvial loam sand soil, (similar to the black earth). The experimental area comprises 6 plots of 290 m^2 each (1878—1961 = 1000 m^2) which have been yearly fertilized since the start of the experiment, as shown in Table 1.

Table 1

Plan of manuring (annual quantity of fertilizers kg/ha)

Plot	Farmyard manure	N	$P(P_2O_5)$	$K(K_2O)$
Farmyard manure (St_I)	12 000	—	—	—
PK	—	—	24 (40)	75 (90)
NPK	—	—	24 (40)	75 (90)
N	—	40	—	—
Without fertilizer (U)	—	—	—	—
Farmyard manure (St_{II})*	8 000			

* = Laid out in 1893, from 1953 without manuring.
Type of fertilizer: N as ammonium sulphate and lime ammonium nitrate.
K as Kainite.
P as Thomas phosphate.

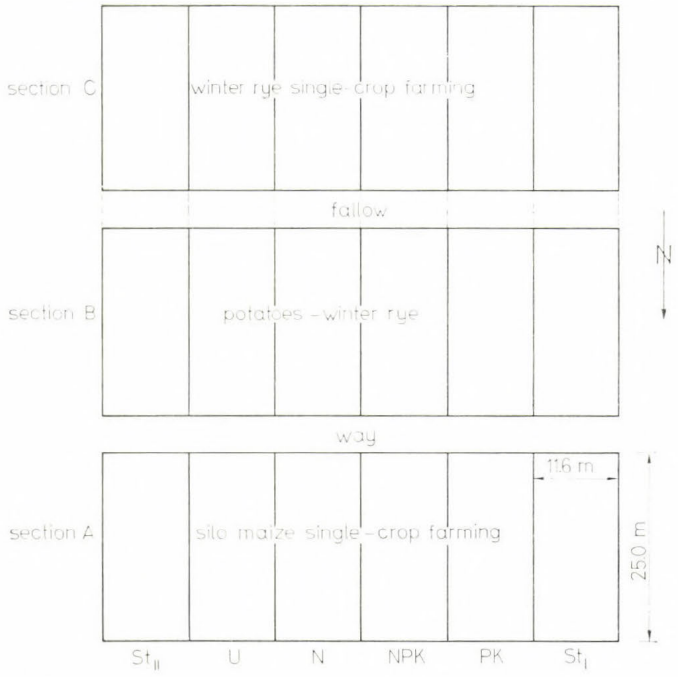

Fig. 1. "Eternal rye-cultivation", Halle. Experimental lay-out

The original experimental area of "Eternal rye cultivation" was in 1962 divided into 3 sections (A, B, C) by reason of increasing invasion by the weed *Equisetum arvense*. The winter rye single crop farming is now limited to section C (Fig. 1). No modifications were made in the fertilizing system in all sections, they are still applied in the original manner and quantity.

EXPERIMENTAL RESULTS

a) *Yields*

In Table 2 the total yield of the winter rye single crop farming is shown for the experimental period from 1879 to 1968.

The total yields obtained on both the farmyard manure plot and the NPK plot are the same. The nitrogen (about 60 kg/ha), annually supplied by the added organic manure, seems to have been utilized by the plants to an extent of about 60%, since the N-supply in form of fertilizers on the NPK plot was about 40 kg/ha. This is also evidenced by the nitrogen balance sheets of this experiment. The low yields of the PK, N and U plots indicated an increasing depletion of mineral substances in the soil. Soil investigations verified these results as well.

The trend of the yields on the individual plots, divided into experimental periods of 15 years each, explains the total yields more distinctly. As evidenced in Fig. 2 only the farmyard manure was able to keep the initial yield

Table 2

Total yields (dry substance) 1879—1968

Plot	Rye	Rel. t/ha	Straw	Rel. t/ha	Total	Rel. t/ha
St_I	199.4	100	376.4	100	575.8	100
PK	128.8	64.6	264.9	70.4	393.7	68.4
NPK	194.4	97.5	386.7	102.7	581.1	100.9
N	155.1	77.8	290.7	77.2	445.8	77.4
U	108.6	54.5	204.4	54.3	313.0	54.4

level approximately. On all the other plots, there is a decrease in productivity, to a higher or lesser degree, in the first experimental period which in the last period adjusts itself to a certain level, according to the special supply of nutritive matter. In the long run NPK fertilizing is not able to keep the yield level of the farmyard manure plot, though at the beginning it shows a certain superiority over the manured plot. The reason for this may be the organic substances and minerals (Ca, nutritious trace elements) which are additionally supplied to the soil with the farmyard manure.

The decrease of productivity in the period from 1954 to 1968, compared to that from 1878 to 1893, with reference to the dry matter total yields on the individual plots, is as follows:

St_I plot = 4.6%
PK ,, = 20.8%
NPK ,, = 16.7%
N ,, = 37.5%
U ,, = 48.3%

The cultivation of potatoes prior to winter rye, performed on the experimental area in section B from 1962, resulted in a distinct increase of the

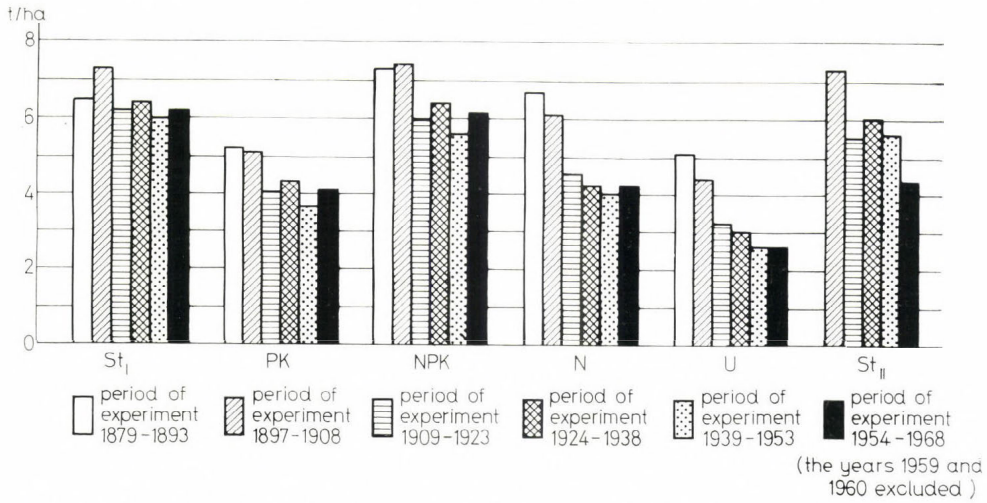

Fig. 2. Annual total yields (dry matter) in average of 15 years' periods

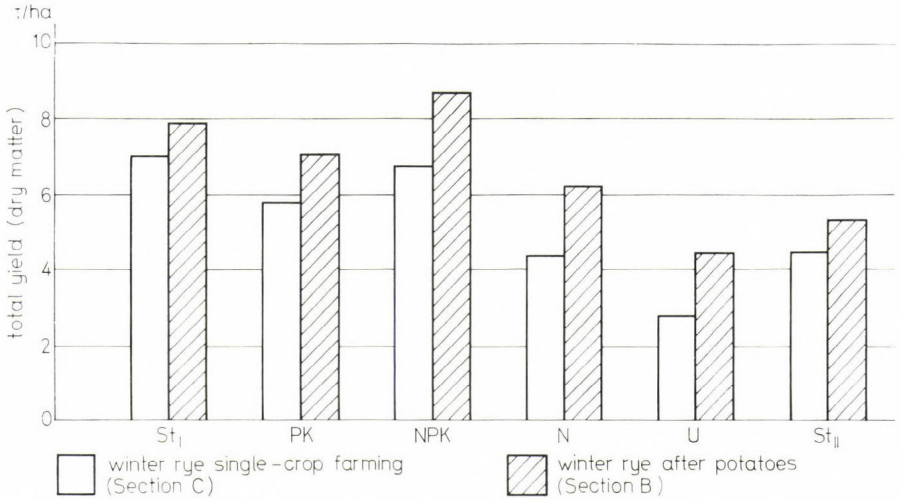

Fig. 3. Crop yield of winter rye in 4 year's average

rye yields, compared with the single-crop farming (Fig. 3). Since the potatoes received the same fertilization as the winter rye, the effect of the preceding crop is quite obvious. The relative high yield produced on the NPK plot, which in the average of the 3 experimental years tested produced yields 10% higher than those of the St_I plot, had not been expected and are not explainable by the present variations of humus and N content of the soil. A similar differentiation shows the potato yields, however, not listed here.

b) *C and N content of the soil*

An important criterion of the fertility of soil is its humus and nitrogen content. Table 3 shows the changes of these characteristics, the individual plots during the experimental period.

These values show the content of humus, compared to the initial state especially in the farmyard manure plot. The increase in C content, ascertained in the plots treated with mineral fertilizers in the last decade, is possibly caused by the influence of flue-dust of the location.

The changes obtained by the individual cultivation measures (i.e., manuring), give evidence of a certain adaptation of the humus level. This is obvious above all on the farmyard manure plot. The highest increase of C content (= 0.40%), seen to be the experimental decades 1—5 while during the decades 6—9 the increase is only 0.17% C, a result which taking into condideration the possible influence of flue-dust might have been smaller. A balance sheet of the farmyard manure plot emphasizes this statement still more (See Table 4).

Table 3

C content (%), N content (mg/100 g soil), and C/N ratio on the top soil (0—20 cm) of the winter rye single-crop farming experiment

Manuring		1878	1929	1958—1967*
St_I	C	1.24	1.64	1.81
	N	—	109	128
	C/N	—	15.0	14.1
PK	C	1.24	1.17	1.34
	N	—	76	80
	C/N	—	15.4	16.8
NPK	C	1.24	1.24	1.37
	N	—	80	86
	C/N	—	15.5	15.9
N	C	1.24	1.23	1.33
	N	—	74	81
	C/N	—	16.6	16.4
U	C	1.24	1.15	1.21
	N	—	71	73
	C/N	—	16.2	16.6

* = Average of samples of 6 years.

Table 4

C balance sheet of the farmyard manure plot

1878 = 1.24% C (= 37.2 t/ha)
1929 = 1.64% C (= 49.2 t/ha)
1958—67 = 1.81% C (= 54.3 t/ha)
Increase in C in the soil = 0.57% (= *17.1 t/ha)*
Farmyard manure, t/ha = 1080 t (= *108 t C/ha)*
(1878—1967)
from it mineralized in % (1878—1929) = 80
from it enriched in % (1878—1929) = 20
from it mineralized in % (1929—1967) = 89.4
from it mineralized in % = 10.6

SUMMARY

Some important experimental results (yield, C and N content of the soil) of the single-crop farming and fertilizing experiment "Eternal rye cultivation" are presented. On account of its long run, the experiment gives insight into the effect of selected measures of manuring on soil and plant.

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EFFECT OF RHIZOSPHERIC MICROORGANISMS ON THE GROWTH AND DEVELOPMENT OF PAPRIKA PLANTS

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In the course of crop production we have to consider — from the view of plant nutrition — the activity of microorganisms living in the soil. The biological processes in the soil are affected by the close association between different microorganisms and higher plants.

Beside the nutrients which are available to plants, the rhizospheric microorganisms affect plant growth and development through their metabolic products. This is justified by those experiments where plants were grown under sterile conditions: Pántos (1956, 1961), Rempe (1961), Krassilnikov (1962), Szegi (1967).

The importance of the physiological processes and products of soil microorganisms from the point of view of plant nutrition is strengthened by the results obtained so far. During the last years a number of important experimental data have accumulated in this problem Ratner (1963) Krassilnikov (1954), Pántos (1961), Horváth (1970).

The most important question is to study the connection between soil microflora and soil fertility. Care must be taken to investigate in detail the biochemical changes and at the same time to keep in mind that the final result ought to be have the highest and best quality yield through the complicated processes of plant nutrition.

MATERIAL AND METHODS

Our aim was to study the effects of rhizospheric microorganisms — using them as inocula — on the growth and yield of paprika plants. The experiment was set up in the Station of Budatétény of the Horticultural Research Institute in an MZG type glasshouse with paprika plant variety: Cecei 12 C/D. The benches in the glasshouse were divided into plots with wooden panels and plastic material (Fig. 1). The soil layer was 20—25 cm deep. There were 4 replicates and 6 treatments. The plots 1.7 m^2 surface area arranged in random blocks. The plants were transplanted one by one and were 15 cm apart in the row. The twin row distance was 40 + 20 cm. The following treatments were applied, in which 28 paprika plants were transplanted in each plot.

a) Inoculation with mixed culture of bacteria isolated from rhizoplane and rhizosphere. Soil sterilized by steam.

b) Inoculation with mixed culture of bacteria and actinomycetes isolated from rhizosphere. Soil sterilized by steam.

c) Inoculation with mixed culture of bacteria and actinomycetes isolated from rhizoplane and rhizosphere. Soil sterilized by steam.

d) Inoculation with mixed culture of bacteria and actinomycetes isolated from rhizoplane and rhizosphere. Non-sterilized soil.

e) Non-sterilized (natural) soil.

f) Soil sterilized by steam.

The plants in the course of transplantation were inoculated with microorganisms. The evaluation was done by variance analysis.

RESULTS

At the end of two years' experimentation the results show that soil sterilization and the use of rhizospheric microorganisms as inocula, were very effective on the growth of paprika plants and at the same time their yielding ability rose. The plants in sterilized soil kept their green colour till the end of the growing season, but in non-sterilized soils, they started to change in colour from green to yellow and decreased in growth even if they had the same inocula (Figs 2 and 3). Taking the yields into consideration we found that the results were different according to treatments. The highest yield was obtained when the plants were grown in steam-sterilized soil inoculated with a mixed culture of rhizospheric bacteria and actinomycetes. As it is represented by the data of Table 1,

Table 1

Crop yield of Cecei C/D kind of paprika

Treatments	Green crop kg/1.7 m²	
	1968	1969
1	37.69	24.38
2	37.34	27.58
3	38.64	28.22
4	30.68	23.44
5	24.24	20.57
6	36.31	26.63

plants yielded the best results in treatment № 3 (inoculation with mixed culture of bacteria and actinomycetes isolated from rhizoplane and rhizosphere — soil sterilized by steam). The treatments № 1, 2, 3 (inoculation+ soil sterilization) gave higher crop than that of № 4, which represented inoculated non-sterilized. The unpublished results of the third year gave similar results.

In the growing season we made observations on the crop, as regards plant protection too. We ascertained that on sterilized soil, the plants were free from viral and fungal diseases and the soil was free from weeds too. In the unsterilized plots some TMV infected plants were found.

On the basis of the above results there was a positive effect due to the treatments on yield (quantity and quality) and average fruit weight.

Fig. 1. Plot arrangements on benches

Fig. 2. Differences in development between paprika plants grown in steam-sterilized and unsterilized soil, inoculated with microorganisms

Fig. 3. Differences in development and colour between paprika plants grown in steam-sterilized and unsterilized soil inoculated with microorganisms

The values were justified by the significant differences. It should be remarked that to achieve high and the best quality yields, the cooperation among scientific researchers in microbiology, and biochemistry as well as special growers is necessary.

SUMMARY

From the results it is obvious that the steam-sterilized soil inoculated with rhizospheric microorganisms proved to have a yield-increasing effect. Paprika plants grown in unsterilized soil also gave higher yields after they were inoculated with rhizospheric microorganisms. The highest yield was obtained when the plants were grown in steam-sterilized soil and inoculated with a mixture of culture rhizospheric bacteria and actinomycetes.

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THE ROLE OF PHYLLOPLANE FUNGI IN THE EARLY COLONISATION OF LEAVES

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Leaves become colonised by fungi almost from the first opening of the bud. The early colonists are to be found on the leaf surfaces, and the term "phyllosphere fungi" was coined by Last (1955) and Ruinen (1956) to describe these organisms. Kerling (1958) suggested that "phylloplane" should be used when referring to the fungi on the leaf surface. This would be consistent with the use of the term rhizoplane for root surface organisms.

Leaf surface fungi have been recognised for a very long time: in 1866, de Bary described *Dematium pullulans* as a fungus commonly occurring on the surface of plants, while in 1887, he referred to work by Zopf on "the soot dew which is found in the form of black fuliginous coatings covering parts of living plants" (p. 249), and to Pasteur's statement about "brown-walled cells which are found on succulent fruits" (p. 271). However, these fungi have only come in for close scrutiny relatively recently, and even in 1961, Ruinen could refer to this habitat as a "neglected milieu".

The most frequently reported fungi on the surfaces of deciduous leaves in temperate climates include the yeasts and a comparatively limited range of *Deuteromycetes*, some of which are the imperfect states of *Ascomycetes* whose perfect states may develop at a later stage in the decomposition of the leaf. Amongst the filamentous *Deuteromycetes*, the most frequently encountered fungi include *Alternaria tenuis*, *Aureobasidium pullulans* (= *Pullularia* = *Dematium*), *Botrytis cinerea*, *Cladosporium herbarum* and *Epicoccum nigrum*. The incidence of these and other fungi on leaves has been comprehensively reviewed by Last and Deighton (1965).

MATERIAL AND METHODS

Leaves of *Acer pseudoplatanus L.* and of *Typha latifolia L.* were regularly collected. Discs were cut from the leaves and were washed in 30 changes of sterile water to remove loosely adhering propagules. Aliquots of water were plated out after each washing to determine the point at which no further propagules were being removed, following the technique of Harley and Waid (1955). The leaf discs were then dried between sheets of sterile filter paper, and plated out on each of two media, potato dextrose agar (P.D.A.) and cellulose agar (C.A.) which was prepared as described by Pugh and Eggins (1962). The plates were incubated at either 10 °C or 25 °C with 25 leaf discs on each medium at each temperature.

RESULTS

1. Effect of Temperature and Isolation Medium

The most commonly isolated fungi are listed in Table 1, together with their frequency of occurrence, which is expressed both as the percentage of leaf discs which yielded the fungi, and as the level of occurrence of the fungi within the total number of isolation.

When considering the temperature relationships of the individual fungi, it can be seen that *Alternaria* and *Aureobasidium* were both more frequently isolated at 25 °C than at 10 °C. This was especially noticeable on *Acer*. *Botrytis* was almost equally abundant at both temperatures, while *Cladosporium* and *Epicoccum* were isolated more often at 10 °C than at 25 °C. These patterns of isolation occurred on both host plants. The effect of temperature was also important in the relative importance of these fungi when their abundance is expressed as a percentage of the total number of fungal isolations. Thus, *Alternaria* and *Aureobasidium* not only occurred on more leaf discs at 25 °C, their proportion of the total mycoflora also increased. The other fungi formed a larger proportion of the total at 10 °C.

Table 1

The occurrence of phylloplane fungi on Acer and Typha leaf discs incubated on two media and at different temperatures

Incubation temperature medium	10 °C				25 °C			
	C. A.		P. D. A.		C. A.		P. D. A.	
	% discs	% isol.	% discs	% isol.	% discs	% isol.	% discs	% isol.
I. *ACER*								
Alternaria tenuis	4.2	2.1	0.3	0.1	24.1	9.7	4.7	1.6
Aureobasidium pullulans	—	—	41.6	16.9	—	—	95.0	32.1
Botrytis cinerea	11.4	5.7	8.3	3.9	11.9	4.8	7.5	2.5
Cladosporium herbarum	89.8	45.4	97.3	39.5	75.0	30.2	77.5	26.1
Epicoccum niprum	40.3	20.2	49.6	20.2	36.3	14.6	41.6	14.0
Total number of discs	280		300		360		360	
Total number of isolations		560		739		894		1070
II. *TYPHA*								
Alternaria tenuis	9.5	3.4	3.8	1.3	9.0	3.6	15.2	5.0
Aureobasidium pullulans	—	—	36.1	12.6	—	—	40.9	13.6
Botrytis cinerea	6.6	2.4	11.9	4.2	7.1	2.8	11.9	4.0
Cladosporium herbarum	68.5	24.7	66.6	23.2	57.1	22.7	61.4	20.0
Epicoccum nigrum	82.3	29.7	64.7	22.5	66.6	26.5	64.7	21.5
Total number of discs	210		210		210		210	
Total number of isolations		581		602		528		632

The effect of the type of medium on the frequency of isolation was most noticeable for *Aureobasidium*, which was not recorded on C. A., although it was abundant on the discs which had been plated out on P. D. A. *Cladosporium*, which occurred frequently on both media, was somewhat more often recorded on P. D. A. The other species all tended to be more frequently recorded on C. A. *Aureobasidium* and *Cladosporium* were relatively more common on *Acer*, while *Epicoccum* was isolated more frequently from leaves of *Typha*.

The isolations recorded in Table 1 were made mainly during the autumn and winter months, when the ambient temperatures generally were low. The more frequent isolation of species at the lower incubation temperature may reflect some change in their competitive status at 10 °C, while *Alternaria* and *Aureobasidium* are favoured at 25 °C. All of these phylloplane fungi have been shown to be able to survive and grow at low temperatures: some of the records are listed in Table 2. It would be very interesting to study further the inter-relationships of these fungi at different temperatures, and the relative frequency of the isolation of fungi from soil and natural substrates when different incubation temperatures are used. It appears to be most important to include an incubation temperature near the ambient temperature prevailing when the samples were collected as well as the normal laboratory temperature which is generally used. This is usually higher than the soil temperature, and may produce results which do not give a true impression of what is actually happening at the ambient temperature.

Table 2

Low temperatures recorded for the growth of fungi which occur in the phylloplane

Fungus	Substrate	Temperature	Reference
Alternaria tenuis	Frozen foods	5 °C	Gunderson (1961)
	in culture	—2 °C	Togashi (1949)
Aureobasidium pullulans	fruit pies	5 °C	Gunderson (1961)
	wood pulp	1 °C	Cooke (1960)
Botrytis cinerea	frozen foods	5 °C	Gunderson (1961)
	spore germination	— 2 °C	Togashi (1949)
Cladosporium herbarum	meat in cold storage	— 6 °C	Brooks and Hansford (1923)
Epicoccum nigrum	leaf litter	3 °C	Mulder (unpublished)

2. Enzyme Production of Phylloplane Fungi

It can be seen from Table 1 that, with the exception of *A. pullulans*, the common phylloplane fungi were isolated on C. A., where their growth was accompanied by a clearing of the medium. This shows that these fungi, in the laboratory, are able to produce cellulase, and this indicates that

they have the potential to decompose cellulose in the field. Other workers have reported that the fungi which are common in the phylloplane produce other enzymes, and some of the more important of these enzyme systems are listed in Table 3. The reported production of cutinase by *Botrytis*, and of pectinase by *Aureobasidium*, *Botrytis* and *Cladosporium* would appear to be particularly important in this ecological group of fungi.

Table 3

Some important enzymes produced by fungi which occur in the phylloplane

Fungus	Enzyme System	Reference
Alternaria tenuis	cellulase	Siu (1951)
	protease	Porter (1966)
Aureobasidium pullulans	pectinase	Wieringa (1956)
	constitutive enzymes for sugars	Clark and Wallace (1958)
Botrytis cinerea	cellulase	Siu (1951)
	cutinase	Linskens and Haage (1948)
	pectinase	Brown (1934)
	enzymes for the utilisation of mannose, glucose, fructose and glycerol	Ampuero (1966)
Cladosporium herbarum	cellulase	Siu (1951)
	pectinase	Wieringa (1956)
Epicoccum nigrum	cellulase	Siu (1951)

DISCUSSION

The initial fungal colonists on the surfaces of leaves are ideally positioned to exploit the underlying tissues at the first opportunity. The possession of enzyme systems by *Botrytis cinerea* which enable it to decompose cutin and pectin, together with the pectinolytic abilities of *Aureobasidium* and *Cladosporium* allow these fungi to penetrate the leaf and to begin the breakdown of the middle lamellae, and thus the disintegration of the leaf tissues. The other enzyme systems, and particularly the production of cellulase, are then able to begin the attack on the cell wall materials before the dead leaf has been incorporated into the soil. At a later stage, however, the highly competitive soil saprophytic fungi normally dislodge the earlier colonists and continue the decomposition of the leaf.

In addition to the part played in the early colonisation of the leaves, the phylloplane flora may also have another, economically important role while the leaf is still alive and actively assimilating: Last and Deighton (1965) have discussed the part which these fungi may play in relation to leaf pathogenic fungi. Thus "the population of *Pullularia (Aureobasidium) pullulans* and *Cryptococcus* spp., which can decompose pectin and lipids may make leaf surfaces more readily wetted and, in so doing, prematurely increase the availability of nutrients to parasites". Conversely, they point out that

the leaf mycoflora "may act as scavengers 'mopping up' energy sources, which otherwise might stimulate the growth of plant parasites".

More recently, Van den Heuvel (1969) has shown that the inoculation of bean leaves with a spore suspension of *Aureobasidium pullulans* a day before or simultaneously with a spore suspension of *Alternaria zinniae,* significantly reduced the level of infection of the leaves. He suggested that the presence of *A. pullulans* acted like a fungicide or a fungistatic substance, and that this effect may be caused by creating a nutrient deficiency in infection drops or by inducing the leaves to produce inhibitory substances.

The phylloplane mycoflora can therefore be seen to be important while the leaves are living. Through their ability to initiate the decomposition of cutin, pectin and cellulose, they are also important during senescence and after the death of the leaves.

SUMMARY

The fungi on the surfaces of leaves of *Acer pseudoplatanus* and *Typha latifolia* which were commonly isolated on potato dextrose agar and cellulose agar include *Alternaria tenuis, Aureobasidium pullulans, Botrytis cinerea, Cladosporium herbarum* and *Epicoccum nigrum.* Differences in the pattern of distribution of these species were found when leaf disc samples were incubated at different temperatures, and on the two media. *Aureobasidium* was not isolated on cellulose agar, but the other species cleared this medium showing that they produce cellulase. The enzyme production of these common leaf surface fungi has been reviewed. The production of cutinase, pectinase and cellulase as well as other enzymes has been related to the role which these phylloplane fungi probably play in the colonisation of the leaf. Their role in relation to infection of leaves by plant pathogenic fungi is also discussed.

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SECTION II

INTERACTIONS BETWEEN HERBICIDES AND MICROORGANISMS

Symp. Biol. Hung. 11, pp. 337—347 (1972)

INTERACTIONS OF SOIL MICROBES AND PESTICIDES

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Pesticides are developed to act in space and time; they are designed to kill, to inhibit or to repel; they are toxic and reactive, and they are introduced into our environment in millions of kilogrammes each year.

With only a few exceptions pesticides are organic compounds, but they do not contribute measurable amounts to the organic matter of the soil in a direct way. Broad spectrum biocides, however, turn a high percentage of living organism into dead organic matter.

Pesticides in general are used for the control of pests and diseases of plants and animals, as well as for the regulation of undesired growth in populations of individuals. Within the approximately 150 groups of chemicals (Table 1) our main interest concentrates on herbicides, insecticides, and fungicides, which are produced and used at an average ratio of 4 : 2 : 1.

Table 1

Survey of pesticides used in terrestrial and aquatic environments

150 major groups of chemicals representing
Acaricides
Poisons and repellents for fishes
Poisons and repellents for birds
Poisons for Amphibians
Poisons for Reptiles
Poisons and repellents for Invertebrates
Poisons and repellents for Mammals
Insecticides
Nematocides
Bactericides
Fungicides
Algicides
Herbicides
Growth regulators
Defoliantia

Interactions between the toxicants and the soil biosphere can occur in two ways: pesticides affect soil microbes, and pesticides are degraded by soil microbes. Both aspects must be seen in conjunction with the main-

tenance of soil fertility as well as with the prevention of hazards to public health. In the following some of the recent results in this area will be presented.

PESTICIDES AFFECTING SOIL MICROBES

There are numerous contributions to this topic available in the literature. In fact, 350 papers had been published before 1963 (Domsch 1963), and the number has about doubled since. This should be acknowledged as a convincing proof for the responsibility and awareness of all people engaged in pesticide research.

On the other hand, it is surprising that the same topic "Pesticide behaviour in soil" is tackled again and again without efficiently increasing our knowledge and understanding. Admittedly, there are some major difficulties, including the extreme diversity of pesticides, soils and microbial populations. But this is not the key for explaining an apparently unsatisfying situation. What soil biology urgently needs is the *quantification* of specific activities of soil organisms, so that the components of the soil ecosystem can be weighed against each other. Today we are still in the position of using a mostly descriptive methodology for the interpretation of functional relationships.

Table 2

Possible effects of pesticides in the soil ecosystem and alternatives for pesticide applications

Pesticides

Target organisms	*Non target organisms*
Alternatives	*Secondary effects* (microbes)
No chemical control;	Reduction of total numbers
specific chemical	Reductions in large groups
control	Reductions on the species level
	Inhibition of global activity
	Inhib. of metabolic activities
	Inhib. of biomass production
	Interruption of nutrient cycles
	Interr. of ecological associations
	Interr. of ecological regulations
Additional effects:	
Pollution of soil,	Stimulation of global activity
water, air; residues	Delay of humus decomposition
in plants and animals	Inhib. of undesired organisms

In Table 2 a provisional survey is given for some of the alternatives for pesticide applications and secondary effects on soil microbes that must be critically weighed *before* an investigation of the system should be started. Four examples derived from Table 2 will illustrate the problem:

1. Low crop quality due to critical amounts of pesticide residues must prohibit pesticide application. In this case a decision about alternatives must be made at an early stage. Consequently, there is no need for an investigation of side-effects on soil microbes.
2. It is known that nitrification is strongly inhibited by many pesticides. On the other hand, the "value" of nitrification for plant nutrition is extremely small. Consequently, the rank of the criterium "nitrification" should be reevaluated.
3. It can be found that total numbers of microbes are drastically reduced by a certain pesticide, while essential soil metabolic activities are still functioning in the same system. Consequently, the higher rank of processes of decomposition, transformation, and production must take precedence over numerical shifts.
4. A high percentage of fungal units is usually eliminated from the soil after fungicide application. With the exception of a few determinations of fungal biomass production in arable soils, the relative importance of fungi as compared with bacteria is still obscure. Consequently, too much emphasis on soil fungi in such cases seems inadequate.

It is evident that an analysis of more or less isolated aspects of microbial activity under the influence of pesticides was the dominating approach to the problem in the past (Alexander 1969). To promote progress in this field, we feel that the complexity of a given terrestrial ecosystem must be critically analysed, system components with the highest degree of sensitivity must be identified, and possible interruption of the energy flow within the ecosystem must be documented.

Research in soil biology should refrain from simply producing data with no interpretation of the actual significance and relevance, leaving the parts of the puzzle for others to compose. We also should refrain from using terms like biodynamic relationship, functional aspects, nutrient cycling, soil metabolism unless we start introducing suitable parameters now. Back in the laboratory, we know well enough that relatively simple models must be chosen as a first step to simulate natural ecosystem. We have made an attempt to study the secondary effects of a fungicide under the following points of view (among others):

a) Do catabolic activities within the carbon cycle have a different degree of sensitivity?

b) Can the restitution of a physiological equilibrium be measured in the soil?

The experimental conditions were as follows: air dried soil, particle size 2—1 mm, remoistened at the beginning of the experiment; incorporation of defined organic substrates (glucose, pectin, chitin, xylan, cellulose, cutin), measurement of O_2-uptake in the absence and presence of captan (N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide) at different concentrations. Calculation of respiration rates and total O_2-consumption.

The percentage of non-oxidized substrate was calculated in the equilibrium state, i.e. when the respiration rates (O_2-uptake/t) had reached a constant level under all experimental conditions. It can be seen from Fig. 1 that substrates which require uniformly distributed enzyme systems for oxidation (glucose, pectin, chitin) are not seriously affected by 500 ppm

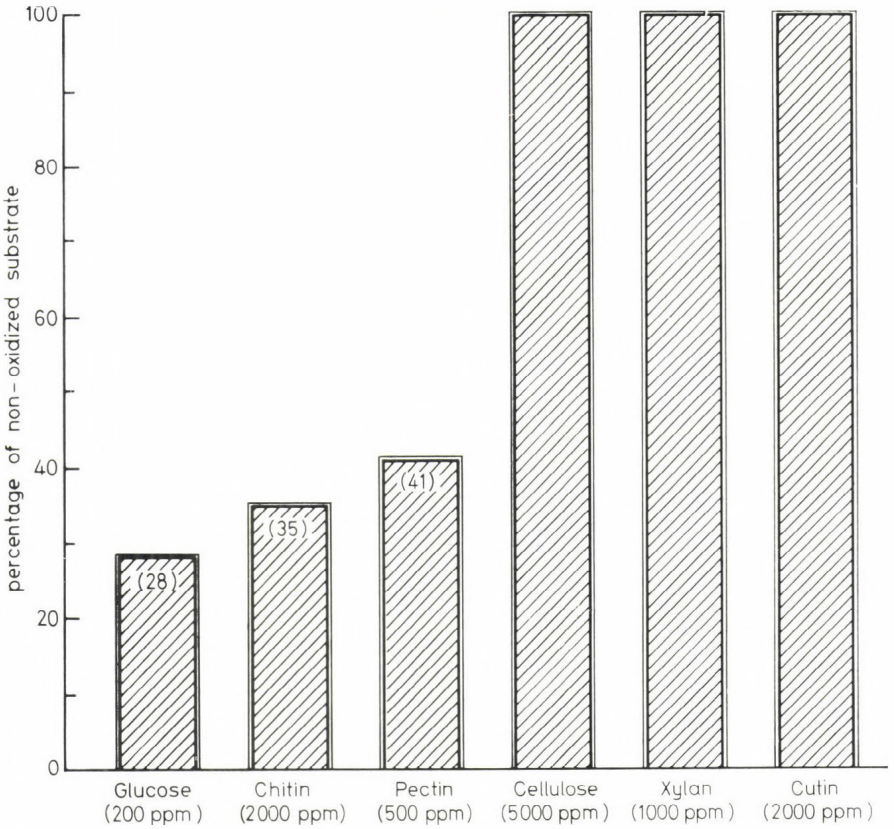

Fig. 1. Influence of 500 ppm captan (active material) on the oxidation of 6 different carbonaceous substrates

Table 3

Influence of 500 ppm captan (active material) on the lag-phases for maximum utilization of 6 different carbonaceous substrates

Time deficit

Application rate of captan (ppm)	Substrate	Delay of maximum utilization (h)
500	Glucose	15
500	Chitin	30
500	Pectin	45
50	Cutin	230
100	Xylan	>400
200	Cellulose	370

of the fungicide. On the other hand, cellulose, xylan, and cutin remain untouched for periods of up to 6 weeks.

Critical concentrations of captan that will permit a delayed utilization of the 3 "blocked" substrates, are 50 ppm for cutin, 100 ppm for xylan and 200 ppm for cellulose respectively. Again, the lag-phase for the glucose, chitin and pectin is of only short duration (Table 3). The restitution of the physiological equilibrium is achieved within reasonable periods of time. If one considers the stress that is put upon microbes by unfavourable environmental conditions (drought, flooding, freezing, heat), the conclusions drawn from temporary inhibitions caused by pesticides should not be exaggerated. Apparently arable soils supporting a highly diversified microbial population have a well developed biological buffering capacity (Domsch 1968).

PESTICIDE DEGRADATION BY SOIL MICROBES

Persistence of pesticides in the soil after they have functioned as a measure for pest control is not wanted in most cases. There is a strong demand for biodegradable chemicals. The process of developing "third generation insecticides" is world-wide, and research has turned to the relations between chemical structure and biodegradability (Kearney et al. 1970).

While microbes are indeed a powerful means of detoxication, a pesticide exposed to the environment is also subject to a number of non-biological degradation mechanisms. At the present time, a good deal of information is available on adsorption, volatilization, translocation, diffusion, photodecomposition, chemical reactions with soil constituents and uptake by plants and soil animals. It remains to be demonstrated to what extent microbes participate in the overall pesticide degradation under varying conditions.

Soil microorganisms perform a number of enzymatic reactions in the process of pesticide degradation. These reactions include oxidation, reduction, ester hydrolysis, dealkylation, dehalogenation, ring hydroxylation and ring cleavage. Pesticides may serve as carbon, aromatic nitrogen or energy sources or they may merely represent substrates for co-metabolic activities (Alexander 1967). It would be highly desirable to have pesticides degraded to a point where no residual toxicity is left, but this is true for only a few pesticides. It therefore should be borne in mind that the terms in use do generally not imply complete degradation, detoxification, or decomposition. The experimental approaches to elucidate pathways of degradation include the use of natural soil samples, enrichment cultures, pure cultures, and enzyme preparations. The system with the lowest order of complexity is generally preferred, since the requirements for biochemical work are very well met. At the same time synergistic effects which are very likely to occur in soil systems are neglected. It is not well enough recognized that in some instances a change from aerobic to anaerobic conditions or a succession of two or more organisms will promote degradation considerably.

In the following, a few examples are chosen from the literature to demonstrate principles of degradation rather than to give a complete survey.

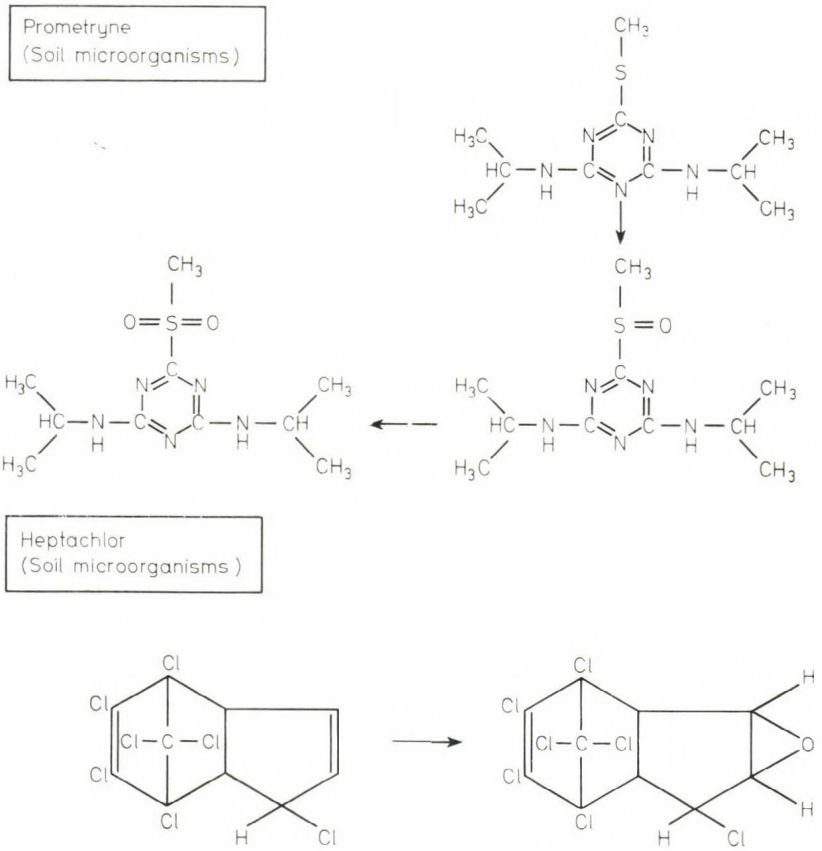

Fig. 2. Oxidation of prometryne and heptachlor

Oxidation (Fig. 2). The oxidation of prometryne (2-methylthio-4,6-bis (isopropylamino)-s-triazine) by non-identified soil microorganisms was reported by Gysin (1962) with the sulphoxide and the sulphone as first degradation products. — The formation of dieldrin from aldrin, and of heptachlor epoxide from heptachlor is based on the capability of many microorganisms to convert isolated double bonds into corresponding epoxides (Menzie 1969). — The beta-oxidation of 2,4-dichlorophenoxyalkanoic acids is very well documented (Gutenmann et al. 1964).

Reduction (Fig. 3). The reduction of nitro groups has been observed in a number of pesticides. Lichtenstein and Schultz (1964) identified aminoparathion in soils with high microbial densities after incubation with parathion (O,O-dimethyl-O-p-nitrophenyl phosphorothioate). — The conversion of PCNB (pentachloronitrobenzene) into pentachloroaniline seems to be in the scope of many soil microbes; *Streptomyces aureofaciens* has been used in the work of Chacko et al. (1966). — The nitro-substituents of trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) and re-

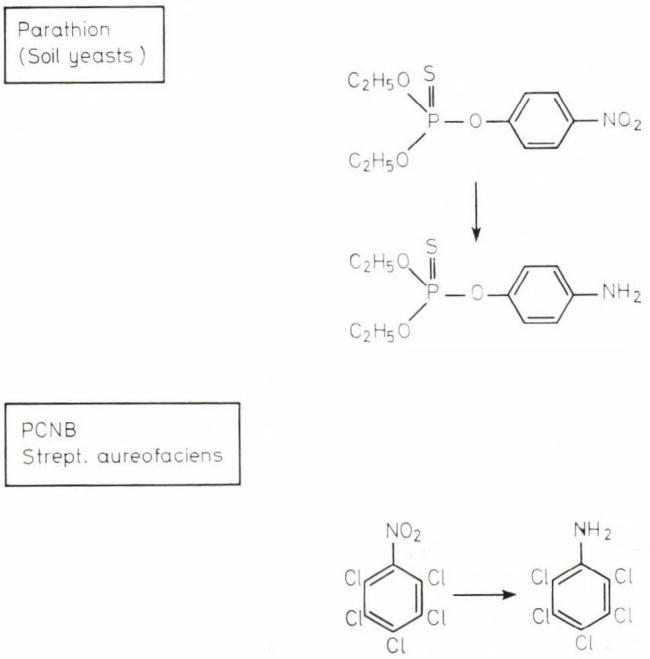

Fig. 3. Reduction of parathion and PCNB

lated compounds are also subject to reduction to amines (Probst and Tepe 1969).

Ester hydrolysis (Fig. 4). Besides the well-known chemical hydrolysis of phosphate insecticides there are several reports on the enzymatic attack of ester linkages. — A pathway for degradation of CIPC (isopropyl N'-(3-chlorophenyl) carbamate) was proposed by Kearney et al. (1967) with 3-chloroaniline, isopropyl alcohol and CO_2 as the preliminary metabolites. — An almost classical example is the conversion of the non-toxic herbicide sesone (sodium 2,4-dichlorophenoxyethyl sulphate) into 2,4-D with 2,4-dichlorophenoxyethanol as an intermediate (Vlitos 1953).

Dealkylation (Fig. 5). The N,O, or S linkages between alkyl groups and the carbon skeleton are in general subject to dealkylation, while C—R substituents seem to be relatively stable (Kearney and Helling 1969). The metabolic degradation of simazine (2-chloro-4,6-bis (ethylamino)-s-triazine) is introduced by a N-dealkylation mechanism with the formation of 2-chloro-4-amino-6-ethylamino-s-triazine (Kearney et al. 1965); it very likely proceeds to the final removal of both ethyl groups, dehalogenation and ring hydroxylation. — In mixed cultures the dealkylation of the herbicide chloroxuron (3-(4-)p-chlorophenoxy(-phenyl)-1,-1-dimethylurea) proceeds via the metabolites 3-(4-chlorophenoxy)phenyl-1-methylurea and 3-(4-chlorophenoxy)phenylurea (Geissbühler et al. 1963).

Dehalogenation (Fig. 6). The process of enzymatic dehalogenation is an essential part of the detoxification of chlorinated hydrocarbon insecticides.

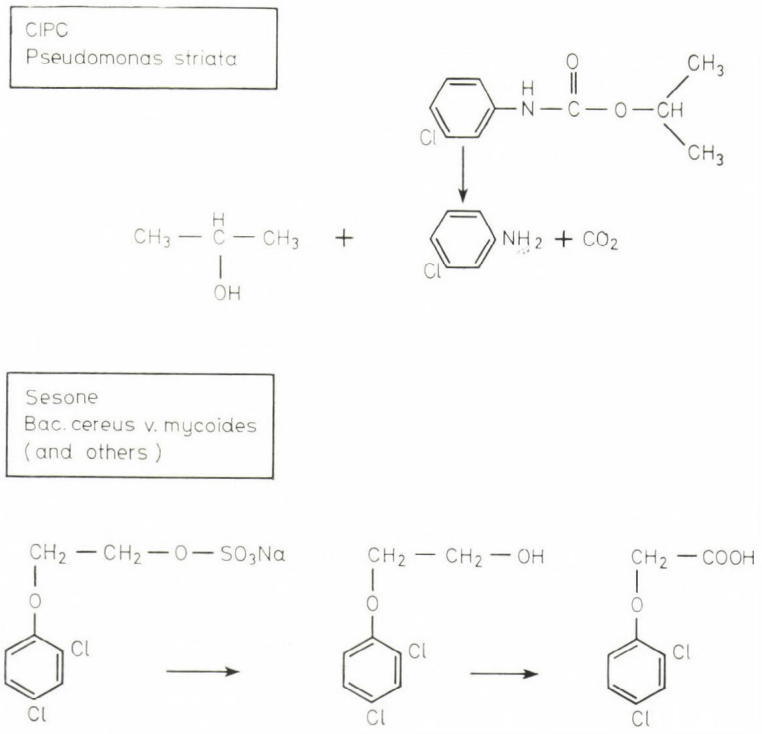

Fig. 4. Ester hydrolysis of CIPC and sesone

So far no reports are available on the dechlorination of molecules in the dien-group, but many microorganisms convert DDT (1,1-dichloro-2,2-bis-(p-chlorophenyl)-ethane) to DDD (Plimmer et al. 1968). According to Allan (1955) several bacteria appear capable of an almost complete dechlorination of HCH (hexachlorocyclohexane) to benzene and monochlorobenzene. Dehalogenation of Dalapon (2,2-dichloropropionic acid) in vitro was demonstrated by an enzyme preparation of an *Arthrobacter* sp. (Kearney et al. 1964). The proposed pathway includes the formation of 2-chloroacrylate and 2-chloro-2-hydroxypropionate. It finally yields pyruvate, which comes very close to an ideal detoxification product.

Ring hydroxylation and *ring cleavage* (Fig. 7). Microorganisms capable of introducing hydroxy groups in the process of metabolizing phenolic compounds are well adapted to aromatic ring hydroxylations. The first step of MCPA degradation involves the introduction of OH-groups in either the 5- or 6-position, followed by the production of 5-chloro-3-methylcatechol. Cleavage of the catechol yields α-methyl-γ-chloromuconic acid (Gaunt and Evans 1961; Faulkner and Woodcock 1965). — The pathway of 2,4-D detoxification follows similar routes. — Further examples for an enzymatic ring cleavage are reported for the insecticide diazinon by synergistic action of two microorganisms (Gunner and Zuckerman 1968) and for carbamyl, a methylcarbamate insecticide (Kaufman 1970).

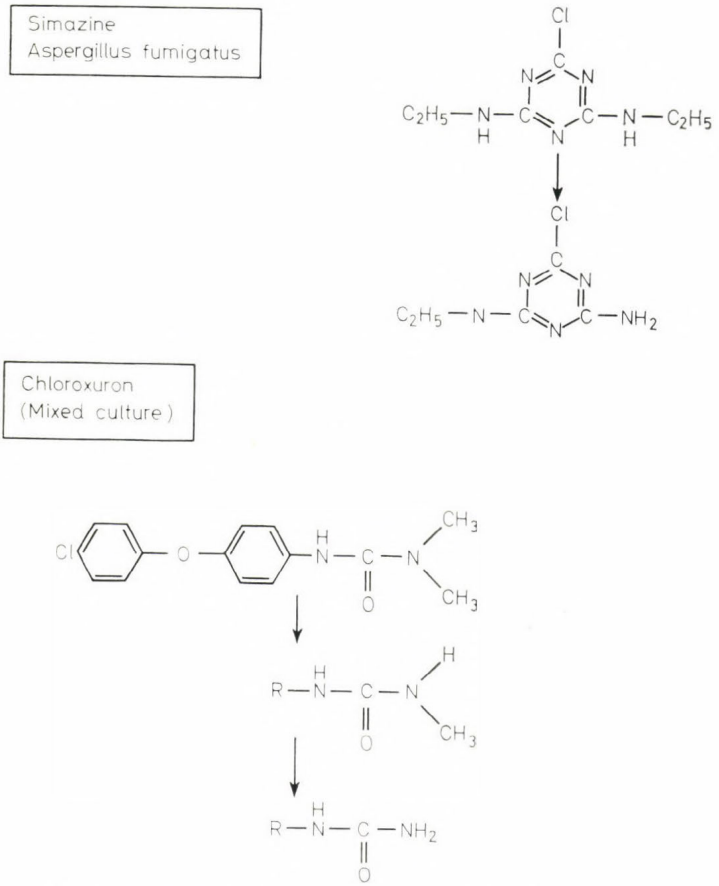

Fig. 5. Dealkylation of simazine and chloroxuron

It becomes almost immediately evident that there is a wide gap between the valuable biochemical work that has been done in pure cultures with mostly biodegradable pesticides and the elucidation of some urgent problems as they occur in the soil environment. One of the most conflicting results seems to be the apparent persistence of some pesticides which are principally biodegradable. The following possibilities should be considered:

Micropopulation:	No capacity for adaptation
	Growth conditions not met in soil
	Growth requirements not met in soil
Pesticide:	Not available to microbes
	Not tolerated by microbes
	No enzyme induction in microbes
Soil:	Physical conditions unfavourable
	Chemical conditions unfavourable
	Changing conditions required

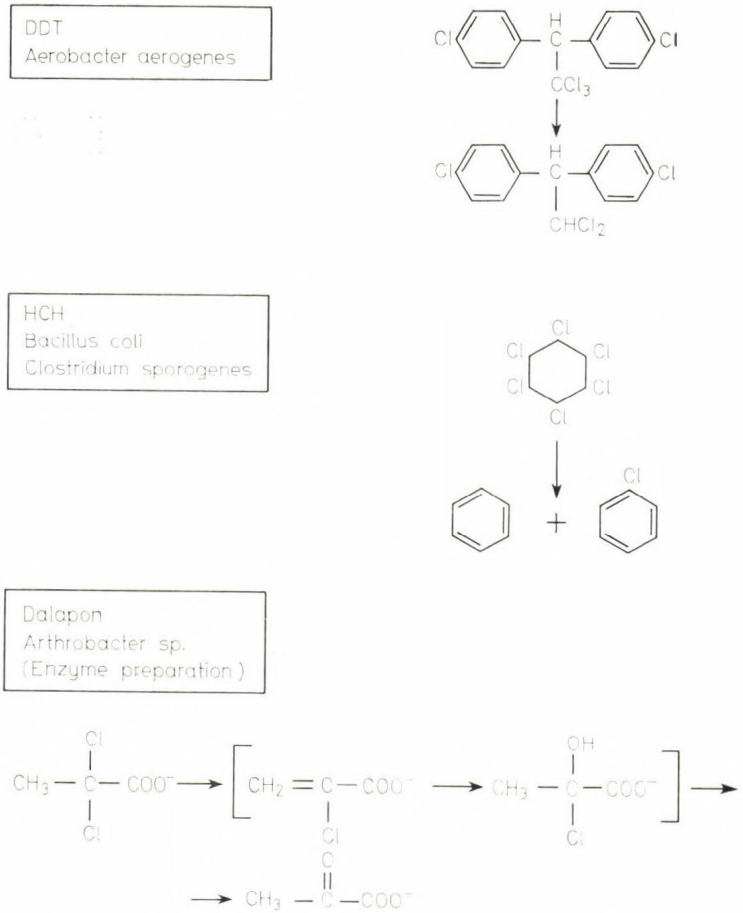

Fig. 6. Dehalogenation of DDT, HCH and dalapon

Let us hope that all efforts will be made in the future to solve the more important problems as they arise from pesticide application. Modern technology has revolutionized agriculture; it should be a matter of public concern to keep all aspects of this progress under control.

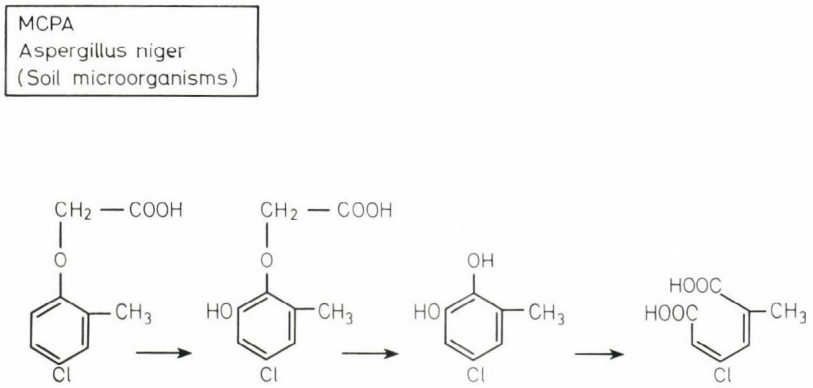

Fig. 7. Ring hydroxylation and ring cleavage of MCPA

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EFFECT OF A FEW HERBICIDES ON THE DECOMPOSITION OF CELLULOSE

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Rapid progress of chemical plant protection demands the use of newer chemical materials for the control of the plant and pests. The pesticides which have been applied in agriculture have increased by leaps and bounds both in their quality and quantity. Thus for example the quantity of pesticides which has been in the past decade increased tenfold and it is very likely that it will increase even at a faster rate. On the basis of the above, the study of the effect of the chemicals used in agricultural practice on the ecosystem of soils assumes a greater importance. Since the micro-organisms living in the soil yield an important part of the ecosystem it is essential to study soil microbes and pesticides both from the theoretical and practical point of view.

Many articles have been accumulated in the journals dealing with the effect of pesticides on biological processes in the soil. Kiss (1967) concluded from the weight-loss of cellulose introduced into the soil as to the effect of the different doses of the herbicides on the soil microflora.

He established that with the increase of the applied doses of Hungazin PK (2-Chloro-4-ethylamino-6-isopropyl-amino-s-triazine) the biological activity of the soil decreased. Domsch (1970) studied the oxygen utilization of soil samples, to which different organic materials (glucose, pectin, chitin, xylan, cutin) as well as Captan (N-trichloro-methylmercapto-4-cyclo-hexane-1,2-dicarboximyde) doses were added.

The respiration investigations proved that some of the listed materials (glucose, pectin, chitin) were mineralized intensively in the presence of 50—200 ppm Captan but the utilization of the three other organic materials was substantially inhibited.

The effect of herbicides on the growth of microscopic fungi living in the soil has been studied by several authors. Colmer (1954), Vedros and Colmer (1959) established that herbicides with DNBP (2,4-dinitro-butyl-phenol) active ingredient strongly inhibited the growth of fungi already in 10 mg/l concentration. Richardson (1957, 1959), Chappel and Miller (1956) observed that the herbicides belonging to the above-mentioned group tested with agar-plate method inhibited the multiplication of different phytopathogenic fungi in 10 mg/l concentration.

EXPERIMENTAL PART

Our basic aim was to study how the cellulase activity of soil, especially the cellulolytic activity of the microscopic fungi isolated from soils is influenced by the herbicides widely applied in plant growing in Hungary.

Soil samples were collected from the ploughed layer of the soils of two Experimental Stations of our institute. One of the soils used was a Hungarian chernozem and the other an alluvial sandy soil. The most important chemical data of these soils are demonstrated in Table 1.

Table 1

The most important chemical data of the investigated soils

Soil type	$CaCO_3$ %	hy 1	pH		Humus %	Total		
			In water	In KCl		K_2O	P_2O_5	N
						mg in 100 g soil		
Chernozem	9.1	2.65	7.5	7.3	3.5	26.8	7.8	2.20
Sand	0.4	0.50	7.0	6.6	0.6	17.0	9.5	1.64

The air-dried soil samples were sieved (pore size 0.5 mm). 250 g of the samples were weighed into porcelain dishes. Each of them received 5 g cellulose powder. Hungazin DT (2-chloro-4,6-bis-(ethylamino)-s-triazine) and Dikonirt (2,4-dichloro-phenol-oxyacetic acid) were added at 0.5, 0.025, and 0.012%. The cellulose powder and the herbicides were mixed with the soil, homogenized then water was added in amount of equal to 60% of the water holding capacity of the given soil. Afterwards the soil was crumbled with water and the samples were put into plastic boxes with a cover which could be locked, but previously an aeration opening was cut on it.

The third herbicide Gramoxone (1,1-dimethyl-1,4-dipyridillumdichlorid) was dissolved in water and added to the soil in the folloving concentrations: 0.5, 0.25, 0.12, 0.06%. Quadruplications were used and 4 parallel samples were taken from every dish during the analysis, therefore data in each case represented the average of 16 samples.

Parallel to the basic experiment, variants without cellulose were prepared with the herbicides using the metho ds outlined above. The dishes were incubated at 28°C for 12 weeks. The evaporated water was replenished every 10th day.

After three months incubation the total organic matter content was determined by Orlov and Grindel's (1967) method, then — after comparing them with the control — the decomposed cellulose was calculated in the different treatments.

The organic matter of soil was destroyed by potassium dichromate and cc. sulphuric acid and the carbon content was determined in a photocolorimetric way and was recalculated to cellulose. As all methods, this also has a disadvantage, namely that the introduction of cellulose could

help the speedy incorporation of humic material and in such way values converted from carbon may be modified to a certain degree.

It was pointed out in an earlier work (Szegi 1965) when the decomposition of cellulose by actinomycetes was studied in the presence of different amounts of Na-humates and parallelly the discoloration degree of the nutrient solution was determined, that one could conclude to the amount of the decomposed humus. But the small decomposition values of humus received there, as well as the application of relatively high doses of cellulose in this work gives us the right to suppose that the main source of error is not this, in the present experiment. The results of the experiment are shown in Table 2.

Table 2

The effect of a few herbicides on the cellulase decomposing activity of the investigated soils

Herbicide	μg active ingredient in 1 g soil	The decomposed cellulose in the % of the measured amount	
		Loose sand	Chernozem
Control	—	76.7	43.9
Hungazin DT	62	80.6	42.7
Hungazin DT	125	72.5	40.5
Hungazin DT	250	75.2	42.1
Hungazin DT	500	74.6	41.3
Gramoxone	125	74.1	46.2
Gramoxone	250	75.5	47.6
Gramoxone	500	64.2	39.7
Gramoxone	1000	60.8	31.2
Dikonirt	87	76.7	44.4
Dikonirt	175	75.9	46.8
Dikonirt	350	72.1	40.1
Dikonirt	700	63.4	43.3

In the second part of this work the sensitivity of different cellulolytic microscopic fungi to Gramoxone, and the different doses of Dikonirt were studied. The experiment was conducted on mineral medium in which cellulose was the only C-source in 2% amount. The pH of the medium was 7. Quadruplications were used. In the case of Dikonirt 0.25, 0.12, 0.06, 0.03 mg/ml medium and in the case of Gramoxone 0.03, 0.012, 0.006, 0.003 mg/ml medium prepared as mentioned was inoculated with the suspension of five-day-old cultures of 20 microscopic fungi. The majority of fungi were isolated from chernozem which was investigated in our earlier work. The fungi belonged to the different species of *Aspergillus*, *Penicillium*, *Nigrospora*, *Fusarium*, *Stachybotrys*, *Verticillium* and *Trichoderma* genera. After three months' incubation, the cellulose which was not decomposed was determined.

Because of the substantial amount of fungal hyphae in the remaining cellulose in the filter which could change the precision of the valuation

for this reason, a method has been worked out to avoid this fault (Szegi 1965). The essence of this method was that after manyfold washing the mixture of cellulose powder and mycelia was dried and the N-content was determined by Kjeldahl method which was calculated to protein and taken as identical with the mycelium weight. The aim of the washing was to remove mucous materials, and the mineral salts, primarily mineral nitrogen. The data of the experiment are given in Table 3.

Table 3

Effect of different doses of some herbicides on the activity of cellulose decomposing fungi

Fungal strains	Control	Concentration of 2,4 D in mg/ml				Concentration of Gramoxone in mg/ml			
		0.03	0.06	0.12	0.25	0.003	0.006	0.012	0.025
	Residual cellulose as a percentage of the added amount								
Aspergillus candidus (L—1)	20.3	19.7	18.6	15.3	10.4	16.4	11.7	8.3	—
Aspergillus ustus	81.2	82.4	66.5	60.8	49.7	67.2	50.3	16.2	—
Penicillium sp. funiculosum series (511)	74.2	73.7	76.9	72.1	69.8	72.4	53.5	26.3	—
Penicillium nalgiovensis (Ksz—11)	79.6	86.1	89.4	80.4	81.5	83.2	42.1	30.7	—
Penicillium sp. purpurogenum series (Ksz—14)	76.5	83.7	87.5	71.4	50.2	66.3	31.5	12.0	—
Penicillium piscarium (L—2)	49.7	50.7	39.8	35.4	27.9	53.4	47.2	14.8	—
Penicillium sp. (L—8)	60.9	64.6	63.8	62.7	53.1	57.2	43.4	19.6	—
Fusarium sp. (L—7)	83.4	68.2	63.5	61.4	59.8	73.4	71.3	48.2	19.6
Fusarium sp. (tn—11)	79.8	65.3	57.4	50.2	40.1	65.6	60.3	39.7	10.2
Fusarium aquaeductuum var. *dimerum* (tn—22)	87.4	73.6	32.4	—	—	76.4	71.2	60.3	50.4
Fusarium avenaceum var. *herbarum* (L—6)	61.2	64.8	63.2	66.5	68.4	55.6	47.4	31.5	—
Fusarium solani var. *argillaceum* (L—12)	75.3	76.4	59.7	39.5	17.2	75.4	73.4	67.5	59.4
Fusarium nivale (L—4)	38.4	39.7	29.3	27.4	26.5	40.2	31.5	18.7	—
Hormodendrum sp. (L10)	39.6	41.4	35.3	37.6	30.9	42.4	40.5	39.6	18.7
Hormodendrum sp. (L—11)	90.4	85.3	80.7	82.5	78.8	90.3	75.7	29.6	15.2
Nigrospora sp.	25.7	25.6	27.2	21.3	19.5	22.3	15.2	13.1	10.9
Stachybotrys atra	47.3	50.4	56.2	49.1	44.3	41.5	36.4	18.4	—
Verticillium candellabrum (L—13)	43.7	41.5	34.8	24.7	18.4	35.1	24.9	11.6	—
Mycelia sterila (L—9)	86.7	71.2	54.6	39.7	30.3	82.3	42.5	11.4	9.3
Trichoderma sp. (111)	80.3	79.5	77.4	71.5	53.2	75.4	72.1	37.5	—

RESULTS AND DISCUSSION

From the experiments mentioned above, one can draw the following conclusions. The data of Table 2 show that the decomposition of cellulose is the most intensive in sandy soil because 75% of cellulose was decomposed during the incubation period. Smaller amount of cellulose was decomposed in the Chernozem soil. It was very interesting to observe in the beginning of the incubation that the sandy soil containing cellulose formed a net, the hypha of different fungi belonged to the *Fusarium* genus. This phenomenon was not observable in the chernozem soil.

According to our opinion in the more intensive cellulose-decomposing activity of fungi in sandy soils could make a basic role to the relatively large amount of the available nitrogen.

As it is demonstrated on the table the manyfold values of Hungazin doses applied in the practice, also did not influence the rate of cellulose decomposition. Neither inhibitory, nor essential stimulative effect could be observed in the Hungazin treatments. It can be supposed that herbicide does not influence substantially the decomposition of the plant residues rich in cellulose in any case of the investigated soil. As regards the Gramoxone its 0.5% dose resulted a visible depression in the decomposition of cellulose. In such a dose the herbicide sprayed onto the surface of plants, could inhibit for some time the decomposition of cellulose and supposedly prevent other microbiological processes from getting partially into the soil not covered with plants.

A smaller inhibitory effect was observed in sandy soil in the presence of 0.25% Gramoxone, which is explainable by the smaller adsorption properties of this soil. The inhibitory effect noticed in the higher Dikonirt treatments compared with the two soil types could be explained also by the adsorption properties. In the other cases the inhibitory effect was not recorded. But no stimulative effect was observed. From the analysis of the results of experiments with pure cultures of fungi, an opposite effect of the two herbicides on the cellulolytic activity of the investigated microscopic fungi was obtained. Thus the relatively high doses of Dikonirt could influence only a few fungal species and the 0.025 mg/ml dose of Gramoxone completely inhibited the growth of the majority of fungi but even the smallest dose of Gramoxone had a marked inhibitory effect compared with the control without herbicide. The same result was recorded by Manninger et al. (1970), where *Rhizobium* and *Azotobacter* proved very sensitive to the Gramoxone dose applied in agricultural practice. It is very interesting that while *Penicillium* and *Aspergillus* species were the most sensitive to Gramoxone, *Fusarium* species were the most resistant. 0.12 mg/ml and 0.25 mg/ml Dikonirt inhibited completely only one strain, the others showed smaller inhibitory effect in the case of the same dose. In addition, smaller doses had a stimulation effect on *Fusarium* species.

SUMMARY

Manyfold doses of Hungazin DT (2-chloro-4,6-bis-ethylamino-triazine) applied in agricultural practice did not inhibit or stimulate decomposition of cellulose in the investigated chernozem and sandy soils. Gramoxone

(1,1-dimethyl-4,4-dipyridillium-dichloride) in 0.5% inhibited markedly the decomposition of cellulose in both soil types, but its inhibitory effect in sandy soil was recorded already in 0.25% Gramoxone dose too. This difference between soil types is explainable by the smaller adsorption capacity of sandy soil. According to our opinion, the same reason is also applicable to the 0.1% concentration of Dikonirt (2,4-dichloro-phenoxy-acetic acid) which exhibited inhibitory effect on the cellulose decomposition only in sandy soil.

Only relatively high doses of Dikonirt inhibited the cellulolytic activity of the 20 representatives of microscopic fungal species and Gramoxone in 0.025 mg/l dose completely inhibited the growth of the investigated fungi.

The most sensitive species to Gramoxone were the *Penicillium* and *Aspergillus* species, while the most resistant were the *Fusarium* ones.

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EFFECT OF SIMAZINE ON THE ANTIBIOTIC ACTIVITY OF *ACTINOMYCETES*

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The effect of Simazine, one of the most frequently used herbicides, on soil microflora has been studied by many authors. In field experiments with Simazine the Polish authors Balicka and Sobieszczanski (1969) have established that it has no effect on soil microflora. In an agar medium, however, the herbicide inhibits the growth of some microorganisms. Koltcheva and Markova (1964) prove that Simazine in concentrations of 1.0—7.5 kg/ha. does not inhibit the development of the actinomycetes. Voderberg (1961) insists that Simazine has a slight effect on the actinomycetes. Zolotukhin (1964) and Steinbrenner et al. (1961) considered that small concentrations of Simazine might stimulate the activity of the soil microorganisms. Studies on the effect of the Simazine on soil microorganisms have been conducted by many others too.

There were no data in the available literature concerning the effect of Simazine on the actinomycete-antagonists and their antibiotic activity.

In view of the important part of the actinomycete-antagonists in the clearing of the soil from phytopathogenic bacteria and fungi, we decided to examine the effect of the Simazine on the soil actinomycetes with antibacterial and antifungal activity.

MATERIALS AND METHODS

The effect of the Simazine on the actinomycete-antagonists was studied on "chernozem smolnitza" (black earth) soil type. The experiment was carried out in three repetitions and four variants with different concentrations of the herbicides: variant I — 5 mg/kg soil; variant II — 10 mg/kg; variant III — 20 mg/kg; variant IV — 100 mg/kg. It was aimed to test together with the normal applied concentrations of the herbicide, others which are many times higher. Simazine was added into the soil in the form of water solution, so that a dampness of 60% of the total moisture capacity should be insured. The samples for the analysis were taken 72 hours after treating the soil at a depth of 0—5 cm and 5—10 cm. As controls soil samples were used which were not treated with Simazine. From dilutions 1 : 1000 of the soil suspension medium were made in a mineral base I (according to Gause 1957). All the isolated actinomycetes were tested for antagonistic activity according to the method of the agar blocks (Egorov 1964) concerning 12 species of microorganisms: *Bacterium tumefaciens, Escherichia coli, Azotobacter chroococcum, Staphylococcus aureus,*

Bacillus cereus, Saccharomyces cerevisiae, Aspergillus niger, Fusarium nivale, Fusarium moniliforme, Fusarium oxysporum, Alternaria tenuis, Trichphyton mentagrophytes.

The effect of Simazine on the actinomycete-antagonists can be determined by the number of the strains with antagonistic activity, isolated from the control and the different variants of the experiment.

The bacteria were cultivated on meat peptone agar (MPA), Ashby and potato agar; the fungi on BA; and the dermatophytes on Sabouraud medium.

RESULTS

The effect of Simazine on the occurrence of the actinomycete-antagonists is presented in Table 1.

Table 1

Effect of Simazine on the spread of the actinomycete-antagonists

Variants	Number of the isolated and tested strains	Number of the antagonists	% of the antagonists
1. Control (untreated soil)			
a. depth 0—5 cm	63	38	60.3
b. depth 5—10 cm	121	62	51.2
2. Variant II (5 mg/kg)			
a. depth 0—5 cm	64	34	53.2
b. depth 5—10 cm	63	31	49.2
3. Variant III (10 mg/kg)			
a. depth 0—5 cm	64	32	50.0
b. depth 5—10 cm	63	30	47.6
4. VariantIII (20 mg/kg)			
a. depth 0—5 cm	68	34	50.0
b. depth 5—10 cm	60	27	45.0
5. Variant IV (100 mg/kg)			
a. depth 0—5 cm	50	26	52.0
b. depth 5—10 cm	53	22	41.5

Simazine does not affect in any significant way the total amount of the actinomycetes, down to 5 cm depth in this soil, but it inhibits the development of part of the antagonists as their amount is reduced. With the increase of the concentrations of Simazine, a gradual reduction of the percent of the antagonists can also be observed.

In the soil at a depth of 5—10 cm the number of the actinomycetes in the soil not treated with Simazine, is about twice as many as that in the treated one. The higher concentrations of Simazine exert a certain inhibiting influence on the actinomycete-antagonists. The actinomycete-antagonists of different test-microorganisms are not inhibited to the same degree by Simazine (Table 2).

From Table 2 it can be seen that Simazine has an inhibiting effect both in high and low concentrations on the antagonists of the *Bacterium tumefaciens*. In the concentrations used in practice (5 mg/kg) Simazine has no inhibiting effect on the antagonists of *Staph. aureus* and *Bacillus*

Table 2

The effect of Simazine on the occurrence of the actinomycete-antagonists on the basis of experiments carried out with different microorganisms

Microorganisms	Number of the tested strains	Number of the antagonists variants				
		Control	I	II	III	IV
1. *Bacterium tumefaciens*	669	39	18	13	10	4
2. *Azotobacter chroococcum*	669	7	7	7	3	5
3. *Escherichia coli*	669	1	1	2	0	2
4. *Staphylococcus aureus*	669	50	52	38	38	34
5. *Bacillus cereus*	669	53	51	37	41	34
6. *Saccharomyces cerevisiae*	669	2	1	1	2	1
7. *Aspergillus niger*	669	11	4	5	4	2
8. *Fusarium nivale*	669	7	6	4	5	5
9. *Fusarium moniliforme*	669	4	4	5	4	7
10. *Fusarium oxysporum*	669	8	7	8	7	7
11. *Alternaria tenuis*	669	18	7	8	9	8
12. *Trichophyton mentagrophytes*	669	10	2	4	9	8

cereus, but with higher concentrations its effect is obvious. The antagonists of the phytopathogenic fungi *Fusarium nivale*, *Fusarium moniliforme* and *Fusarium oxysporum* are inhibited neither by the high nor by the low concentrations of Simazine. Fungi possessing actinomycete-antagonist properties, e.g. *Alternaria tenuis*, are inhibited to the same degree both by low and high concentrations of Simazine.

The distribution of actinomycete-antagonists in the control and the different variants of the experiment are given in Table 3.

Table 3

Distribution of the actinomycete-antagonists in different series

Series	Control	Number of the actinomycete-antagonists variants			
		I	II	III	IV
1. *Lavandulaeroseum*	2	2	3	0	2
2. *Fradiae*	7	4	2	2	1
3. *Fuscus*	0	1	0	0	1
4. *Roseoviolaceus*	3	2	2	3	2
5. *Ruber*	0	0	0	1	0
6. *Helvolus*	2	2	1	1	2
7. *Albus*	2	0	1	0	0
8. *Coerulescens*	12	10	7	4	6
9. *Griseus*	17	14	8	6	2
10. *Nigrescens*	1	1	0	1	0
11. *Aureus*	34	31	26	26	17
12. *Chrysomallus*	5	2	2	2	0
13. *Chromogenes*	6	3	3	3	3
14. *Violaceus*	6	3	6	9	9

The increasing concentrations of Simazine in the soil bring about the gradual diminution of the antagonists from the series: *Aureus, Coerulescens, Griseus* and others, but in some of the series there is no appearance of such an effect or activity.

SUMMARY

1. The examined concentrations of Simazine inhibit to a slight extent the development of the actinomycete-antagonists, both at a depth less than 5 cm in the soil and a depth of 5—10 cm. By increasing the concentrations of the Simazine to 100 mg/kg the antagonists are reduced.
2. The antagonists of the phytopathogenic fungi *Fusarium nivale*, *Fusarium moniliforme* and *Fusarium oxysporum* are inhibited neither by the low nor by the higher concentrations of the Simazine. With the antagonists of the *Bacterium tumefaciens* the reverse process is observed — they show a tendency to be inhibited both by higher and lower concentrations of Simazine.
3. By increasing the concentration of Simazine in the soil, a number of the antagonists from the series: *Aureus, Coerulescens* and *Griseus* is gradually decreased, while with other series there is no evidence of such a phenomenon.

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THE INFLUENCE OF SOME HERBICIDES ON THE DEVELOPMENT AND THE ANTIBIOTICAL ACTIVITY OF *ACTINOMYCETE*-ANTAGONISTS WITH ANTI-FUNGAL ACTIVITY SPECTRA

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The introduction of herbicides in the soil, although in very small doses, can cause alternations in the composition of the microorganisms and can change their normal assimilation processes. There may be observed an activation of some metabolic reactions in favour of others. Charpentier and Pochon (1962) have established that Simazine can serve as a source of nitrogen in the case of some microorganisms. Steinbrenner et al. (1961) conducted laboratory experiments with Simazine and B—6638. Some herbicides in media were added and inoculated with soil-suspension for establishing the changes in the quantitative and species composition of the existing microflora. Mishustin (1964) established that after the phase of inhibition, caused by the herbicides on the microorganisms a stimulating effect, may appear, due to the gradual decomposition of the herbicide in the soil. Geller and Chariton (1961) consider that the herbicides inhibit most of all the nitrogen-fixers and they have less effect on the cellulose-decomposing bacteria. The most susceptible are the fungi and the mildews and the actinomycetes are less affected. Klyuchnikov and Petrova (1960), after treating forest chernozem with herbicides found an increase in the quantity of *Penicillium* and *Aspergillus* and a decrease of *Trichoderma*.

The aim of our work was to establish what influence different concentrations of some herbicides used in this country, have on the development of isolated actinomycete strains and their anti-fungal activity. This is important in order to find products for anti-fungal preparations.

MATERIAL AND METHODS

The effect of five herbicides has been studied: Lasoe, Treflan, Ramrod Semeron and Dymid, which are used in our country, on the antibiotic activity of 43 strains of actinomycetes, the anti-fungal activity of which was established beforehand. The experiment was conducted in four variants, for every herbicide, and for comparison a control was used in which no herbicide was applied. The different variants of the experiment contained gradually increasing doses of herbicides: for the Lasoe — 8, 13, 27, 67/1 media, for Treflan — 8, 16, 32, 80/1.; for Ramrod — 16, 32, 48, 128/1; for Semeron — 8, 16, 28, 96/1.; media. For different herbicides varying concentrations were used depending on the doses used in practice.

The test was done by introducing the herbicides in solid agar medium (mineral 1 according to Gause 1957), on which the actinomycete-antagonists

were cultivated. The effect of the herbicides was determined by comparing the antibiotic activity of the separate actinomycetes strains on the control and the different variants of the experiment in relation to the six kinds of phytopathogenic fungi (*Alternaria tenuis*, *Pythium debaryanum*, *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Verticillium albo-atrum*). The test fungi were cultivated on beer-agar at 26°C. The results of the experiment are indicated in Tables 1 and 2.

RESULTS

A manifold activity of the investigated herbicides has been established. The antibiotic activity of some of the actinomycete-antagonists was decreased and others of it increased. The activity of the herbicide Lasoe was investigated on 15 actinomycete-antagonists. Concerning *Alternaria tenuis* only 3 strains retained their activity on an equal level in the control and in the different variants of the experiment. Two strains diminished their antibiotic activity and 10 strains increased their antagonistic properties under the influence of the herbicide. It is characteristic that both the stimulating and the inhibiting action of the herbicide depends on its concentration. In most cases (6 strains out of 15), an increase in the antibiotic activity was found at lower concentrations of Lasoe. The higher doses (variants III and IV) with some antagonists increased and with others diminished the antifungal activity. Strain No. 22 under the influence of the highest of the tested doses (variant IV) entirely lost its antagonistic activity to *Pythium debaryanum* and *Rhizoctonia solani*. One actinomycete strain can be influenced in such a way as for example strain 25 in variants (I, II and III) increases its fungicide activity towards *Pythium debaryanum* and diminishes it towards *Rhizoctonia solani*. This activity of the herbicide can be explained, if we assume that one strain can produce several antibiotic substances, and the biosynthesis of only one of them is inhibited.

Similar to the activity of the herbicide Lasoe is the effect of the remaining investigated herbicides. It is characteristic that the actinomycete-antagonists, whose antibiotic activity is stimulated by small concentrations of the herbicide (variant I and II), do not exhibit antagonistic activity towards a given test fungus after its treatment with a herbicide if they had no such properties before that.

If we compare the activity of the doses of herbicides used in practice (variants I and II), on *Alternaria* it can be seen that the different herbicides affected differently. In the case of Lasoe the stimulation was predominant. With the herbicides Treflan and Semeron — the inhibiting activity was dominant (with Treflan treatment 8 out of 15 investigated strains diminished their activity; with Semeron 6 out of 12 stems decreased their antagonistic activity). The herbicide Ramrod predominantly stimulated the actinomycete-antagonists (10 out of 12 strains were stimulated). The herbicide Semeron did not (as regards the antibiotic activity 10 of the 15 strains increased in this respects) exert any influence on most of the actinomycete strains with antagonistic activity (from 11 investigated strains — 3 were stimulated, 2 inhibited and 6 were not affected).

Table 1

Effect of the herbicides Lasoe, and Treflan on the antibiotical activity of actinomycetes expressed along the diameter of the inhibitory zone (in mm)

Test fungi	Variants	Lasoe							Treflan						
		Number of the actinomycete strains													
		2	9	10	11	22	24	25	2	9	10	11	15	19	20
Alternaria tenuis	C	28	20	22	25	24	21	34	28	20	22	25	22	23	28
	I	30	20	22	24	20	22	34	29	20	23	30	23	24	0
	II	30	28	22	28	20	24	30	26	21	24	26	24	22	0
	III	33	30	28	28	16	26	30	24	29	24	20	24	23	0
	IV	34	24	23	23	16	24	30	24	18	24	0	26	18	0
Pythium debaryanum	C	21	20	20	16	20	12	18	21	20	10	16	0	13	17
	I	22	20	18	19	17	22	21	20	0	16	16	0	13	0
	II	24	20	16	20	13	18	26	16	0	16	15	0	16	0
	III	24	20	16	19	13	13	40	14	0	17	16	0	16	0
	IV	20	16	15	16	0	16	18	12	0	12	20	0	10	0
Rhizoctonia solani	C	28	20	22	28	26	24	29	28	20	22	28	27	20	22
	I	28	21	26	26	27	20	25	26	23	20	23	23	20	0
	II	20	24	28	26	22	20	20	26	24	20	22	22	19	0
	III	22	24	24	27	21	20	20	25	30	20	21	21	20	0
	IV	14	29	22	30	0	20	18	25	22	17	22	18	18	0
Fusarium oxysporum	C	21	21	20	25	18	13	24	21	21	20	25	27	21	25
	I	22	20	20	28	18	14	26	20	21	20	21	24	20	0
	II	28	21	20	20	20	18	21	19	20	20	20	17	20	0
	III	26	24	20	22	20	19	21	20	18	19	16	16	20	0
	IV	19	20	18	14	22	22	22	20	10	20	12	16	16	0
Sclerotinia sclerotirorum	C	35	28	26	28	20	22	28	35	28	26	28	26	22	15
	I	30	30	30	28	24	21	28	28	28	28	28	26	20	14
	II	30	32	32	31	28	22	30	26	28	29	30	23	21	14
	III	32	34	34	28	28	22	30	24	28	32	34	23	20	15
	IV	20	26	25	30	28	19	28	24	28	27	21	21	19	18
Verticillium albo-atrum	C	30	30	30	33	26	30	30	30	30	30	33	20	20	18
	I	30	26	30	30	26	26	30	28	30	28	30	28	28	20
	II	30	24	27	26	24	25	31	28	32	26	30	28	28	19
	III	28	20	26	18	25	20	30	26	30	24	29	28	28	20
	IV	30	20	22	16	26	20	30	26	29	24	30	30	26	20

C = control.

Table 2

Effect of the herbicides Ramrod and Semeron on the antibiotical activity of the actinomycetes, expressed in diameter of the inhibitory zone (in mm)

Test fungi	Variants	Ramrod								Semeron					
		Number of the actinomycete strains													
		1	2	3	4	8	9	10	11	1	2	3	11	19	20
Alternaria tenuis	C	28	28	20	18	22	20	22	25	28	28	20	25	23	28
	I	30	29	0	20	24	28	30	30	30	28	20	21	23	28
	II	34	34	0	22	24	28	32	34	28	28	21	21	22	16
	III	38	40	0	22	26	28	29	31	28	28	22	14	20	13
	IV	29	29	0	21	26	26	30	27	28	26	22	14	20	12
Pythium debaryanum	C	23	21	0	19	25	20	20	16	23	21	0	16	13	17
	I	20	18	0	18	18	22	20	17	24	17	0	16	14	18
	II	20	18	0	16	18	24	20	18	25	16	0	16	13	13
	III	18	17	0	12	14	18	20	20	19	15	0	16	13	0
	IV	16	14	0	10	13	18	20	20	16	15	0	12	12	0
Rhizoctonia solani	C	26	28	0	13	26	20	22	28	26	28	0	28	20	22
	I	27	28	0	14	20	28	21	24	26	26	0	28	24	16
	II	30	30	0	13	18	26	20	24	25	26	0	28	24	14
	III	34	30	0	13	—	26	20	22	25	21	0	28	24	0
	IV	30	30	0	12	—	26	20	20	25	21	0	27	20	0
Fusarium oxysporum	C	20	21	0	13	14	21	20	25	20	21	0	25	21	25
	I	22	26	0	18	14	20	24	26	24	21	0	29	26	28
	II	22	24	0	18	20	21	24	26	23	20	0	23	21	12
	III	31	20	0	14	23	20	22	30	20	20	0	16	18	10
	IV	16	12	0	14	20	21	21	21	16	16	0	0	16	10
Sclerotinia sclerotiorum	C	30	30	10	18	27	28	26	28	30	30	10	28	22	23
	I	30	30	0	20	26	29	26	26	30	29	18	28	23	20
	II	30	29	0	19	25	30	25	25	30	27	22	30	23	18
	III	29	28	0	18	26	31	26	23	30	28	24	36	21	16
	IV	29	29	0	18	26	20	26	20	30	28	20	29	20	16
Verticillium albo-atrum	C	30	30	30	28	—	30	30	33	30	30	30	33	22	18
	I	32	30	28	27	—	30	30	30	30	24	20	30	23	18
	II	31	30	28	27	—	28	30	30	30	22	21	26	22	16
	III	31	29	27	24	—	21	30	26	30	21	20	20	22	14
	IV	30	30	26	24	—	21	30	26	30	21	20	20	22	14

The established activity of the studied herbicides on the actinomycete-antagonists was confirmed by the results received in the additional testing of all the 43 strains of actinomycetes with antifungal effect. The results from all these tests cannot be presented fully here and for this reason we shall confine ourselves in giving only some of the results in the form of two tables attached.

In the course of the experiments we established that some noticeable differences in the growth and the development of the actinomycetes are not to be observed and only their activity changed.

1. The influence of the studied herbicides on the antibiotic activity of the actinomycetes with antifungal activities varied. Some of the actinomycetes strains were stimulated, others were inhibited, and others were not influenced at all.

2. Both the stimulating and the inhibiting influence of the herbicides depended on their concentrations. In most cases the increase in the antibiotic activity was observed on the effect of small doses of herbicides (variants I and II), but in some strains there was a stimulating influence both in the lower and the higher doses (variants III and IV).

3. A given actinomycetes strain can be influenced by the herbicides in such a way that towards some of the test fungi it increased its antagonistic activity while towards others it diminished it.

4. From a microbiological point of view, the best herbicides are Lasoe and Ramrod, because they have practically no negative influence on the soil actinomycetes with antifungal effect. On most of the tested strains they have a stimulating effect. The herbicides Treflan and Semeron decrease about half of the actinomycete strains with a fungicide activity to *Alternaria tenuis*.

SUMMARY

The influence and the effect of the herbicides: Lasoe, Treflan, Semeron — 25 and Ramrod on the growth and the development of actinomycetes possessing different degrees of antifungal activity was studied.

The effect of the different concentrations of the herbicides on actinomycetes was established which have antagonistic properties in relation to phytopathogenic fungi and other microorganisms.

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ACTION OF SIMAZINE ON THE ANTIBIOTIC ACTIVITY OF MICROSCOPIC SOIL FUNGI

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The tendency to the constant rise in the number of herbicides used in practice led to the problem of studying their influence on the soil microflora. Most of the studies in this field are concerned with the influence of the herbicides on soil bacteria, while their influence on the microscopic fungi and moulds has not been sufficiently investigated.

Klyuchnikov and Petrova (1960) report that 2,4D inhibits the development of *Trichoderma* in the rhizosphere of young oak trees. At the same time the authors have observed an increase in number of the representatives of the *Penicillium sp.* and a certain increase of the *Aspergillus sp.*

Rodriguez-Habana et al. (1968) established that the effect of herbicides depends on the concentration in which they are used. The authors established that Atrazine introduced into the soil in a dose of 8 g/1 kg stimulates the growth of *Sclerotium rolfsii*, while in greater doses inhibits the fungus growth. The investigations of other authors (Ivanova 1968; Bakalivanov 1969; Fields, Hemphill, Virág 1959; Pántos et al. 1964; Voderberg 1961) show that some of the herbicides inhibit and others have no effect on the different kinds of microscopic fungi and moulds.

In the soils of Bulgaria the fungal-antagonists are very wide-spread and their influence and importance for the clearing of the soils is very great, especially for the clearing of the soil from phytopathogenic microorganisms (Bakalivanov 1962; Vlahov 1964). Taking this consideration into account, we have set as our task to analyse and study the influence of Simazine on the microscopic soil fungal-antagonists of bacteria, fungi and dermatophytes.

MATERIAL AND METHODS

The effect of the herbicide was studied on the fungal-antagonists in "chernozem smolnitza" (black earth smolnitza) soil type. The test was conducted in four variants, treated with different doses of Simazine: Variant I — 5 mg in 1 kg of soil; Variant II — 10 mg in 1 kg of soil; Variant III — 20 mg in 1 kg of soil; Variant IV — 100 mg in 1 kg of soil. As a control soil not treated with herbicide was used. The test samples were taken for analysis 48 hours, 72 hours, and 7 days after the introduction of the herbicide into the soil, from two soil layers: at a depth of less than 5 cm and 5—10 cm. The isolation of the microscopic fungi was achieved on beer agar inoculated with 0.1 ml soil suspension (dilution 1:100). The antago-

nistic properties of the isolated fungi were studied according to the method of the agar blocks (Egorov 1964) on 12 types of microorganisms: *Bacterium tumefaciens*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Azotobacter chroococcum*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Fusarium nivalae*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Alternaria tenuis* and *Trichophyton mentagrophytes*. The influence of Simazine on the antagonistic properties of the soil fungi was established by comparing the number of the antagonists with the control and the different variants of the experiment. The test microorganisms were cultivated on the following nutrient media bacteria on MPA, Ashby and potato agar, the phytopathogenic fungi — on beer agar and dermatophytes — on the medium of Sabouraud. Identification of the fungal-antagonists on specific level was achieved by means of the determining key of Gilman (1957) and Kursanov (1956).

RESULTS

It was established that with rise of the concentration of the Simazine and the continuation of its action there is a diminution of both the total number of the microscopic fungi and the number of the antagonists (Table 1). Simazine shows depressing effect on the antagonists irrespective of the degree of their antagonistic activity. After treating the soil with 100 mg of herbicide for 1 kg of soil there are only individual antagonists found there. The inhibiting effect of Simazine is more strongly expressed

Table 1

The effect of Simazine on the occurrence of the fungal-antagonists

Number of the variants	Number of isolated and examined strains	Number of antagonists			
		Total number	Strong	Medium	Weak
1. Control (not treated with herb.)	83	37	11	14	12
2. Variant I (5 mg/kg)					
a) after 48 hours	62	21	7	7	7
b) after 72 hours	7	2	0	2	0
c) after 7 days	4	4	0	2	2
3. Variant II (10 mg/kg)					
a) after 48 hours	35	11	0	3	8
b) after 72 hours	4	0	0	0	0
c) after 7 days	8	2	0	0	2
4. Variant III (20 mg/kg)					
a) after 48 hours	21	10	5	5	0
b) after 72 hours	10	3	1	1	1
c) after 7 days	8	5	0	4	1
5. Variant IV (100 mg/kg)					
a) after 48 hours	12	3	1	2	0
b) after 72 hours	9	1	0	1	0
c) after 7 days	5	1	0	1	0

Note: Strong antagonists — inhibitory zone more than 30 mm in diameter. Medium antagonists — inhibitory zone 20—30 mm in diameter.
Weak antagonists — inhibitory zone 1—20 mm in diameter.

in the surface layer of the soil at a depth of less than 5 cm (Table 2). In this depth of the untreated soil with herbicide, more microscopic fungal-antagonists are observed than in the bigger depth of soil (5—10 cm). In both of the soils treated with different doses of Simazine almost an equal number of antagonists were observed at a depth less than 5 cm and in that at a depth of 5—10 cm.

Table 2

Number of the fungal antagonists from different depths 48 hrs, 72 hrs and 7 days after the introduction doses of Simazine into the soil

Variants	After 48 hours		After 72 hours		After 7 days	
	Depths					
	to 5 cm	5—10 cm	to 5 cm	5—10 cm	to 5 cm	5—10 cm
1. Control (not treated with herbicide)	23	14	23	14	23	14
2. Variant I (5 mg/kg)	10	10	0	0	1	1
3. Variant II (10 mg/kg)	10	11	1	0	2	2
4. Variant III (20 mg/kg)	5	5	—	1	2	3
5. Variant IV (100 mg/kg)	1	0	1	2	0	0

The inhibiting effect of Simazine on the fungal-antagonists, varied according to the genera. Especially strong is the effect on the representatives of the species of *Penicillium* and *Aspergillus*. The antagonists of the *Trichoderma* are inhibited only by the highest concentrations examined of the herbicides (100 mg/kg). There is no effect of the herbicides on fungi *Trichoderma lignorum*, *Penicillium notatum*, *Aspergillus flavus*, *Aspergillus terreus*, *Paecilomyces varioti* and *Fusarium cephalosporium* which show a strong antibacterial and anti-fungal activity. There is no influence on their antibiotic activity even with the highest concentration of the tested herbicide. These antagonists are, however very rarely found in investigations of soil types and they make only 30% of the total amount of microscopic fungi, possessing antagonistic properties.

SUMMARY

1. Simazine exerts an inhibiting effect on the soil fungal-antagonists, both in low and in higher concentrations. This inhibitory effect was stronger the higher the concentration of the herbicide and the longer of the time of its activity.
2. The inhibiting effect of Simazine was established in both the examined soil depth but it is stronger in the surface soil at a depth less than 5 cm.
3. The fungal-antagonists from the different species are not equally sensitive to the effect of Simazine. Its effect is especially strong on the representatives of the species: *Penicillium* and *Aspergillus*.
4. Simazine has no effect whatsoever on the antagonists of the species *Trichoderma lignorum*, *Penicillium notatum*, *Aspergillus terreus*, *Paecilomyces varioti* and *Fusarium cephalosporium* which exhibit a strong antibacterial or anti-fungal activity. There is no inhibiting effect on their antibiotic activity either with low or higher concentrations of herbicides.

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BEHAVIOUR OF SOME HERBICIDES USED FOR WEED CONTROL IN VINEYARDS ON DIFFERENT SOILS

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Afalon and Arezin are recommended at present for chemical weed control in vineyards. They have the disadvantage of not being able to kill in a satisfactory way besides the monocotyledonous weeds. *Convolvulus arvensis* one of the most widespread weeds of the vineyards. In areas treated only with Afalon, the weeds develop to such extents that it reaches the conditions of territories getting no chemical weed control at all.

The 3 years long experiments carried out at the Research Institute for Viticulture and Oenology has shown that a combined substance containing 40% p-chlorophenoxy acid-i-propylester, 30% Aktikon PK (90% 2-chlor-4-ethyl-amino-6-isopropylamino-s-triazin) and 30% supplementary materials kill, besides other weeds, *Convulvulus* too. Due to results of the experiences the mentioned herbicide must be administrated every second year besides Afalon to kill the *Convolvulus* too.

MATERIAL AND METHODS

Pot cultures were used in the experiments. Soils used were collected from the Experimental Stations of the Research Institute for Viticulture and Oenology (Budapest) situated in Kecskemét—Miklóstelep and in Tarcal. The water content of the soil was adjusted in case of the first soil (a sandy-soil) to 10 and 25% of its maximal water-holding capacity where in the second soil to 25 and 40% of the water-holding capacity, and both were held at this level during the whole experiment. The herbicides were added according to the practical dosage of 18 kg per hectare and their tenfold amount: at its calculation the surface of the culture pots was taken into consideration. Watery suspensions of the herbicides were made and in this form they were added to the surface of the soils in order to secure their homogeneity. Samples were taken of the same quantity (6.28 cm^3) from 3 parts of every culture pot and the samples were shaken in 100 ml of Sørensen's buffer solution during 15 minutes. The soil solutions were then cleaned from the soil particles through a filter and from every sample 1 ml was taken out with a pipette and was put in a reaction-vessel.

The respiration of the microorganisms was measured by the Warburg-technique.

RESULTS AND CONCLUSIONS

Before using the above-mentioned combination of herbicides a microbiological investigation of the soil must be carried out to be sure about the effect of the herbicides on the soil microorganisms. With this aim the

intensity of respiration of every microorganism found in the soil was investigated to see the effect of the herbicide on this function; furthermore we have examined whether the water-content of the soil does not influence the process under examination.

The two soils of Kecskemét—Miklóstelep and Tarcal were chosen because they are from the most important areas as regards quantity and quality of wine-production. The two soil types were also very different: the first was a loess sandy-soil, the second a more compact one. The water-holding capacity and some characteristic features of the soil were determined.

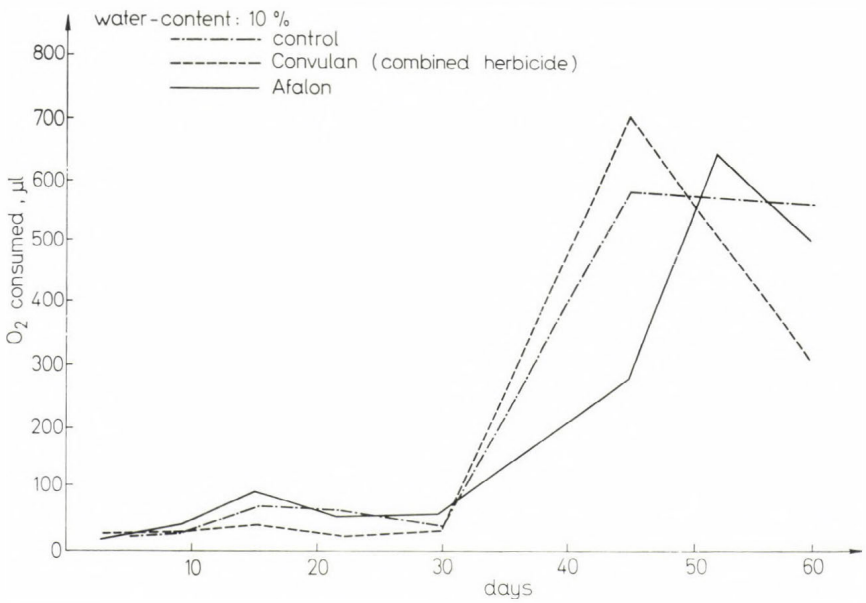
Humus-content of the sandy-soil was 0.30%, its pH value was 7; humus of the loess = 0.79, its pH value = 7.3, value of U. L. P.: 21.1.

The consumption of oxygen was measured every hour and the sum of the result in O_2-content is illustrated by four figures.

After treating the loess soil we did not find a definite connection between the respiration of the microorganisms and the herbicide effects. This can be explained perhaps by the absorption of the herbicide used.

In case of the sandy-soils the two different water-contents did not show any difference in the respiration-intensity of the microbes but the reaction concerning intensity of herbicide-concentration was definite. The data are shown by an axial-system in function of time. In Figs 1 and 2 we present the oxygen consumption measured in the soils used after treatments with herbicides of 0.3% concentration. We can notice, that the two soils of different water-content but getting the same herbicide-concentration hardly

Fig. 1. The effect of 0.3% herbicides (added to the sand containing 10% water) on the respiration of soil microorganisms. Water content: 10%, —·— control, - - - - Convulan (combined herbicide), ——— Afalon



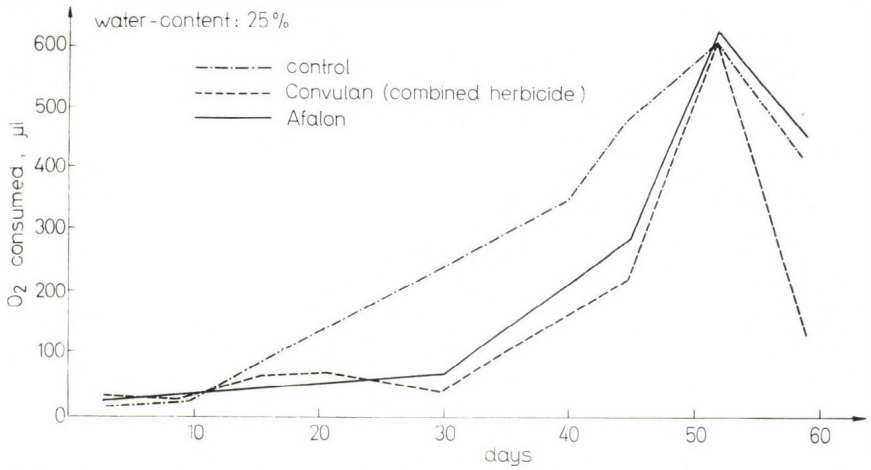


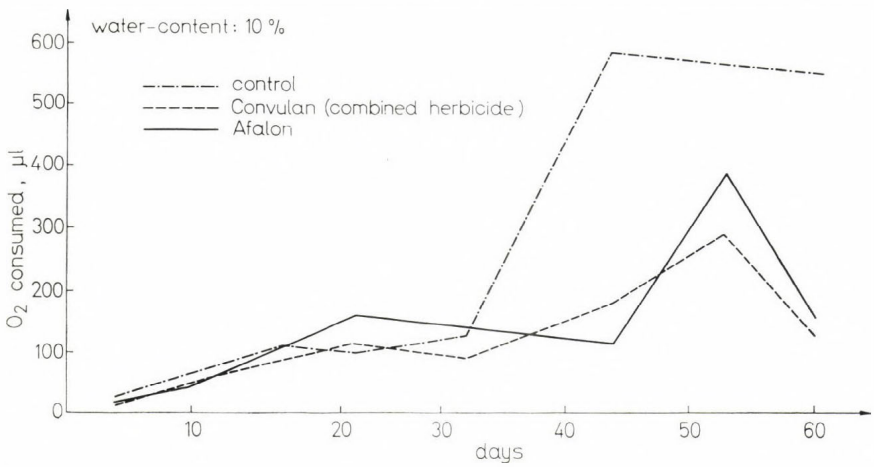
Fig. 2. The effect of 0.3% herbicides (added to the sand containing 25% water) on the respiration of soil microorganisms. Water content: 25%, —·— control, - - - - Convulan (combined herbicide), ——— Afalon

differ from each other. The same is to be seen in Figs 3 and 4 showing oxygen consumptions as affected by the herbicide of 3%.

The difference is striking when comparing the respiration of microorganisms living in sandy-soils of the same water-content with various concentrations of herbicides.

The respiration was definitely inhibited by a higher concentration and this inhibition did not cease after 60 days. It must be noted, that such a high dosage is never used in practice.

Fig. 3. The effect of 3% herbicides (added to the sand containing 10% water) on the respiration of soil microorganisms. Water content: 10%, —·— control, - - - - Convulan (combined herbicide), ——— Afalon



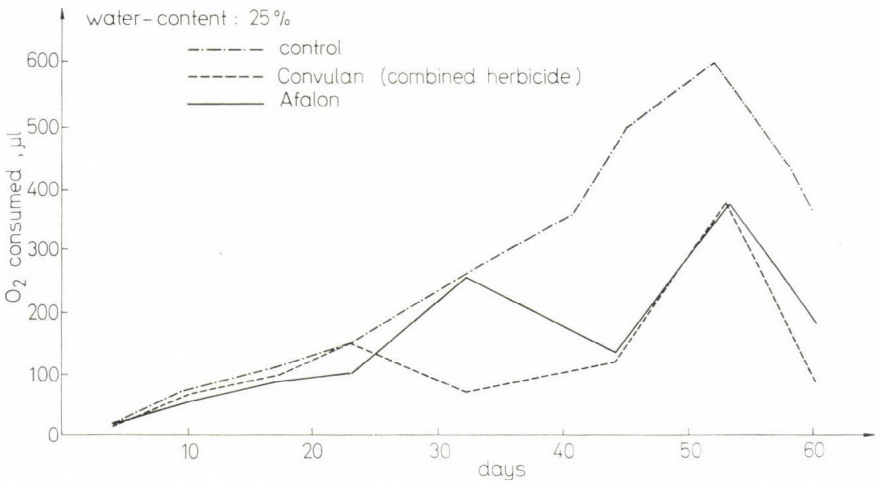


Fig. 4. The effect of 3% herbicides (added to the sand containing 10% water) on the respiration of soil microorganisms. Water-content: 25%, —·— control, - - - - Convulan (combined herbicide), ——— Afalon

SUMMARY

Afalon and Arezin are recommended for weed control at present. They have a disadvantage: besides the control of monocotyledonous weeds, which are very wide-spread in vineyards, the *Convolvulus arvensis* is not controlled by them in a satisfactory way. A combination of 40% p-chlor-phenoxyacetic-i-propylester. 30% Aktikon PK (90% 2-chlor-4-ethyl-amino--6-isopropyl-amino-s-triazin) and 30% supplementary material is able to kill, besides other weeds, *Convolvulus* too. Before using the weed-killers, in the above-mentioned combination its effect must be investigated on the microorganisms of the soil, by means of microbiological soil-examination. We used for our experiments pot-cultures filled with two different soil-types. The first one originated from the Experimental Station situated on the sandy soil of Kecskemét—Miklóstelep of the Research Institute for Viticulture and Oenology (Budapest), the second from the loess soil of Tarcal of the same Research Institute. Respiration of the microorganisms was measured with the Warburg apparatus. After treating the loess we could not find a definite connection between the intensity of respiration of microorganisms and effects of the herbicides. In case of sandy-soils the two different water-contents did not show any difference in intensity of microbe respiration but the effect of the different herbicide concentrations was the same. The data were shown by an axial system in function of time.

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BIOLOGICAL ACTIVITY OF CERTAIN HERBICIDES ON MICROSCOPIC SOIL FUNGI

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The use of herbicides in agriculture is ever increasing. Along with the killing of weeds, negative phenomena occur in the soil with the soil microorganisms and biological processes. Arnold et al. (1966) have shown soil treatments with 2,4-D and herbicides of similar composition to provoke excretion of toxic matter of certain microscopic soil fungi. According to Penner (1967) other herbicides inactivated some enzymes. Certain herbicides were found by other authors (Bakalivanov and Nikolova 1969; Bakalivanov 1969, Bakalivanov and Nikolova 1969b) to cause a quantitative decrease of the useful soil microflora while some herbicides stimulated the microbiological processes in the soil.

The biologic activity of herbicides used should therefore be thoroughly studied with a view to acquire complete knowledge of their effect on soil microorganisms.

MATERIAL AND METHODS

The author analysed the effect of a number of herbicides on 5 widespread microscopic fungi species viz.: *Paecilomyces varioti*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus tamarii* and *Penicillium funiculosum*. The following triazine herbicides were used: Atrazine, Prometryne and Saminol, preparations on the basis of dichlorophenoxyacetic acid: 2,4-D and Dikotex and the ones based on urea as Afalon and Cotoran as well as mixed Semparol (based on triazine with dichlorophenoxyacetic acid). For this purpose 10-day-old pure cultures of the mentioned fungi were incubated on Czapek's medium, containing herbicides in the usual doses used in practice. Analyses were carried out by determining the mycelium of dry weights of these fungi, the intensity of their respiration (CO_2 excretion) and synthesis of the catalase and urease enzymes. The reaction of the fungi culture liquid was also determined with a view to study their acidifying power as affected by the individual herbicide.

RESULTS

Respiration tests with studied fungi showed the inhibition effect of triazine herbicides Atrazine, Prometryne and Saminol on the excretion of CO_2 (Fig. 1). 2,4-D had a similar effect though not on all fungi studied. The other investigated herbicides did not distinctly affect the fungi tested except

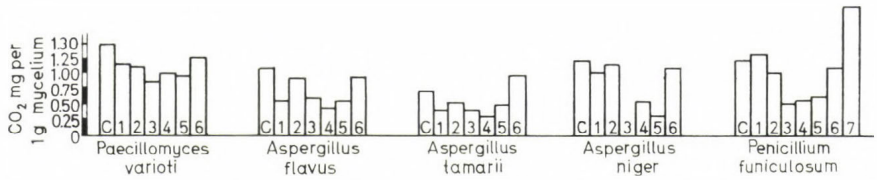


Fig. 1. CO_2 elimination; catalase and urease of certain microscopic fungi, treated with different herbicides (CO_2 mg per 1 g mycelium)

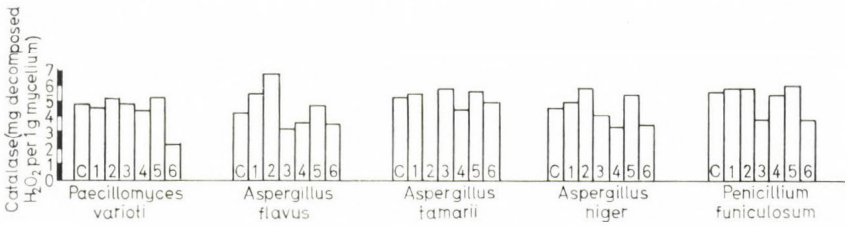
Paecilomyces varioti, on which almost all preparations used had a respiration inhibitory effect. Such an effect on the soil microflora following triazine herbicides (Simazine and Atrazine) treatments was demonstrated in other studies too (Bakalivanov and Nikolova 1969, Bakalivanov 1969). Most herbicides failed to stimulate the respiration of the fungi studied except Afalon which stimulated distinctly the respiration of *Penicillium funiculosum*.

As to catalase enzyme synthesis by the triazine herbicides Atrazine, Prometryne, and mixed Semparol a certain inhibition tendency could be observed manifesting itself to a higher degree against *Aspergillus flavus*, *Aspergillus niger*, *Paecilomyces varioti* (Fig. 2). Only Semparol had a relatively larger field of activity which inhibited nearly all fungi studied. Catalase synthesis tests showed a stimulatory effect of Dikotex particularly on the *Aspergillus flavus*, and *Aspergillus niger* fungi.

Of all fungi studied, synthesis of the urease enzyme was inhibited by the triazine herbicide Atrazine (Fig. 3). Prometryne and Semparol had a clearly expressed inhibiting effect however on *Aspergillus flavus* only which proved to have the highest sensitivity among the fungi studied. A certain trend towards an inhibiting effect on the excretion of urease was manifested only by the 2,4-D herbicide among all fungi tested. During the analyses certain herbicides used were found to inhibit greatly the excretion of the urease enzyme. Thus as affected by the herbicides Dikotex and Saminol, increased enzyme synthesis was most clearly expressed with *Aspergillus tamarii*.

According to pH analyses of the culture liquid of these fungi its reaction was affected to a certain degree by the herbicides used (Table 1). All herbicides having an alkaline reaction, the pH was slightly increased upon addition to the medium. Some more important changes of the pH as affected by determined herbicides occurred after the fungi had developed thus proving that some of the preparations used can modify the

Fig. 2. Catalase (mg H_2O_2 decomposed per 1 g mycelium)



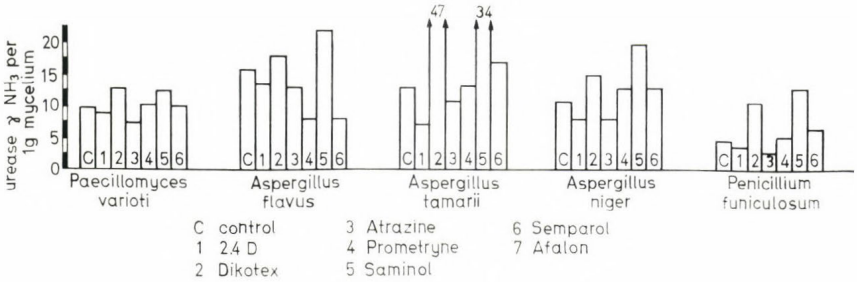


Fig. 3. Urease (NH_3 per 1 g mycelium)

physiology of the fungi as regards the excretion of acids as well. This is an important aspect of the biological activity of the investigated fungi and the breakdown of the organic matter in the soil. As affected by the preparations Dikotex and Saminol, analyses showed a substantial alkalizing of the cultural liquid during development of all fungi examined. This process was most clearly expressed with the *Peacilomyces varioti* and *Penicillium funiculosum* fungi. pH differences of the variants treated with the same herbicides were less marked compared with the control which was still distinct. Only the *Aspergillus niger* fungi were an exception in this respect which probably due to the intensity of their acidifying power, partially neutralized the alkalizing activity of the preparations and did not cause substantial changes in excretion process of the normal acids. The relevant specific reaction of some fungi species was confirmed by the studies of Rodriguez et al. (1967) who noted an increased intensity of acid excretion under the effect of Atrazine.

Studies on the vegetative development of the fungi mycelium showed certain of the treated herbicides to have an inhibiting effect (Table 1). The triazine preparation Saminol proved to have the most markedly expressed inhibiting effect on the mycelium dry weight with all tested fungi. As affected by the development of the *Peacilomyces varioti*, mycelium was reduced by 50 per cent and more. The inhibiting effect of the preparation was less markedly noticeable on the other fungi tested. Significant data were obtained on mathematical systematization of the results of this herbicide. No clearly expressed effect on the proliferation of the mycelium was noted with the remaining triazine preparations Atrazine and Prometryne. A significant stimulating effect of Prometryne was found only with the *Aspergillus tamarii* and *Aspergillus niger* fungi. The fungi mentioned might have been more resistant to the toxic effect of the preparation in this case and therefore had only an irritating effect. As a result some weight increase of the mycelium of the said fungi could be noted. The phenomenon is also attributable to the use of the preparation as a nutrient source. Dichlorophenoxyacetic acid herbicides stimulated the vegetative development of most investigated fungi. A more definite effect was obtained by 2,4-D. The results of its effect were significant in all cases while Dikotex displayed only a low stimulating effect, hence no significant differences were noted with most of the investigated fungi. The same herbicides inhibited the development of the *Aspergillus tamarii* mycelium, attribut-

Table 1

Effect of certain herbicides on pH and vegetative development of certain microscopic soil fungi

Microscopic fungi (species)	Variants	pH of culture liquid		Weight of dry mycelium mg on 20 ml Czapek	Mean error ± m	Safety coeffcient P
		Without fungus	With fungus			
Peacilomyces varioti	Control	4.30	3.30	188	5.7	
	2,4 D	4.85	3.70	234	2.7	4.1
	Dikotex	4.80	9.00	201	4.8	2.8
	Atrazine	5.15	4.00	207		1.3
	Prometryne	4.65	3.55	191		0.7
	Saminol	4.90	8.20	80	7	5.3
	Semparol	4.70	3.85	108	11.7	0.5
	Afalon	7.60	7.55	162	2.5	1.2
	Cotoran	7.65	8.30	158	5.0	2.9
Aspergillus flavus	Control	4.30	6.75	173	7	
	2,4 D	4.85	6.88	240	61	3.1
	Dikotex	4.80	8.03	236	5	3.7
	Atrazine	5.15	7.15	175	8	0.4
	Prometryne	4.65	7.81	87	5.2	0.6
	Saminol	4.90	8.85	162	3.9	2
	Semparol	4.70	8.82	157	8.9	1.5
	Afalon	7.60	7.90	179	2.2	2.6
	Cotoran	7.65	8.35	133	2.9	13.5
Aspergillus tamarii	Control	4.30	5.96	212	4.2	
	2,4 D	4.85	6.34	172	4.3	7.7
	Dikotex	4.80	8.63	187	4.8	4.8
	Atrazine	5.15	6.53	182	3	7
	Prometryne	4.65	6.16	260	3.4	7
	Saminol	4.90	8.12	176	4.3	3.9
	Semparol	4.70	6.93	273	1.7	2.9
	Afalon	7.60	5.10	191	2.0	7.0
	Cotoran	7.65	8.30	186	2.0	10.8
Aspergillus niger	Control	4.30	2.50	189	8.6	
	2,4 D	4.85	2.94	285	5.3	4.3
	Dikotex	4.80	3.50	223	7	0.8
	Atrazine	5.15	3.00	160	2	1.6
	Prometryne	4.65	2.77	244	2	5
	Saminol	4.20	3.40	140	118	4.8
	Semparol	4.70	3.03	199	7.9	0.6
	Afalon	7.60	2.80	179	1.3	7.5
	Cotoran	7.65	2.30	151	1.9	2.0
Penicillium funiculosum	Control	4.30	4.70	182	3.4	
	2,4 D	4.85	5.15	220	2.7	3.0
	Dikotex	4.80	3.05	206	9	1.6
	Atrazine	5.15	3.98	176	1.7	3.7
	Prometryne	4.65	4.13	203	10	2.6
	Saminol	4.90	7.32	130	10	3.8
	Semparol	4.70	4.32	177	10	0.6
	Afalon	7.60	4.70	198	2.6	3.0
	Cotoran	7.65	4.50	174	0.9	1.6

able to the higher sensitivity of this fungus towards these preparations. A similar toxic effect of 2,4-D on the proliferation of certain fungi was observed by Arnold et al. (1966) with slightly increased doses. According to our investigations the usual doses had an inhibiting effect with some more sensitive fungi species and a stimulating one for other fungi. The favourable influence of the dichlorphenoxyacetic acid preparations on the development of the mycelium of the studied fungi is attributable to its physiological activity on certain plant tissues, chiefly manifested by their proliferation (Audus 1963). Most probably this acid has a similar effect with respect to the mycelium of the fungi as well.

Of Urea herbicides used, the Cotoran preparation had an inhibiting effect on the development of the mycelium of almost all analysed fungi.

SUMMARY

1. The triazine herbicides Atrazine, Prometryne and Saminol had an inhibiting effect on the respiration of all fungi analyzed. Some of these preparations, as well as Semparol, inhibited the synthesis of the catalase and urease enzymes of certain fungi. Vegetative development of some of them was affected negatively by Saminol and excretion of urease was stimulated.

2. The dichlorophenoxyacetic acid herbicides 2,4-D and Dikotex had a stimulating effect on the development of the mycelium of most fungi. 2,4-D showed a more distinct effect. A stimulating effect of Dikotex was noted in respect to the catalase synthesis and particularly to that of the urease of certain fungi.

3. Of the urea herbicides Afalon showed a clearly expressed stimulating effect on the respiration of *Penicillium funiculosum*. The Cotoran preparation inhibited the mycelium of all examined fungi.

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INTERACTION BETWEEN SOIL MICROFLORA AND HERBICIDE AGELON

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In the last ten years many studies have been conducted on the interaction between herbicides, applied to the soil, and soil microflora. They indicate two effects of great practical significance. One is alteration of the chemicals through the physiological activities of microflora, leading to the disappearance more or less of the weed controlling agent. Another involves the potential inhibition of the chemicals, either to the total soil microflora or to separate groups of microorganisms, which are essential for soil fertility.

Studying this problem, our investigation up to now are concerned mainly with the influence of herbicides upon soil and rhizosphere microflora (Micev and Bubalov 1969a, 1969b, 1969c; Micev 1970). The experiments described in this paper, were undertaken to study the effect of herbicide Agelon on soil microflora, as well as tolerance and metabolism of herbicides by some isolated bacteria.

MATERIAL AND METHODS

In experiments reported here, the herbicide Agelon was used in doses of 3 kg/ha. The plots planted with maize culture, were located on alluvial soil type. Sampling was carried out twice, in one month interval, from herbicide-treated and untreated plots. The first sampling was taken in May, ten days after treatment with herbicide, and the second sampling in June, one month later. The following groups of microorganisms were investigated: aerobic heterotrophic bacteria, aerobic spore-forming bacteria, actinomycetes, fungi, aerobic N-fixing bacteria and aerobic cellulolytic microflora. Methods and media used are described in our earlier paper (Micev and Bubalov 1969c).

From herbicide-treated soil samples, several characteristic species of *Arthrobacter* were isolated, which were investigated for tolerance and metabolism of Agelon. Since herbicide Agelon consists of a combination of Atrazine and Prometryne, we investigated them separately. The tolerance of bacteria was investigated in two variants, one with 2% and another with 5% added glucose on flat agar media. To both variants herbicides (Atrazine and Prometryne) were added also in concentrations of 0.1%, 0.5%, and 2%, in active ingredient; for controls, the plates without herbicides were used.

Isolated strains of bacteria species which developed in colonies were inoculated with the loop on 3 spots, of such preparated media. Each strain

of bacteria was inoculated on 3 Petri dishes and incubated at 25 °C for 8 days. The developed colonies were afterwards measured. The average size of 9 colonies in cm was taken as the final value. On the basis of growth and average size of colonies the effect of herbicides was estimated, respectively the tolerance of bacteria to various concentrations of herbicides.

Since the *Arthrobacter* No 11 and 23 proved distinct tolerance to the herbicides they were then examined for utilization of herbicides as the sole source of carbon. For this purpose media were used which contained no carbon besides herbicides in concentrations of 0.1, 0.5 and 2%. The utilization of herbicides as a sole source of carbon was estimated on the basis of growth and size of colonies. Media with herbicides, and media with usual sugar only as control, were incubated at 25 °C for 10 days.

RESULTS AND DISCUSSION

In Table 1 are presented the results of the effect of herbicide Agelon (Atrazine and Prometryne) on soil microflora. At first sampling depressive effect of herbicide on actinomycetes, aerobic, cellulolytic microorganisms,

Table 1

The effect of herbicide Agelon on soil microflora

Samples	Number in 000			% of fertile grains		
	1	2	3	4	5	6
Control I	1,200	600	2,120	23	66.0	72.0
II	31,240	2,660	8,280	177	77.0	64.0
Agelon I	4,600	1,710	1,600	44	63.0	58.0
II	21,400	1,950	1,800	220	90.0	66.0

1. Aerobic heterotrophic bacteria
2. Aerobic spore-forming bacteria
3. Actinomycetes
4. Fungi
5. *Azotobacter*
6. Cellulolytic microorganisms

I Sampling on 23.5
II Sampling on 26.6

and in some degree on aerobic N-fixing bacteria are expressed. The other groups (aerobic heterotrophic bacteria, aerobic spore-forming bacteria and fungi) were more or less stimulated. At the second sampling a depressive effect was found in the case of aerobic heterotrophic bacteria, aerobic spore-forming bacteria and aerobic cellulolytic bacteria, whereas fungi and nitrogen-fixers were stimulated. In both samples fungi were stimulated, while actinomycetes were depressed.

From the soil microflora, of the herbicide-treated plots, several characteristic strains of bacteria were isolated, which were then examined in the laboratory for tolerance to Atrazine and Prometryne. As it is evident from Table 2 two species were tolerant: No. 11 and 23.

According to the growth and size of colonies in cm it was evident that strain 11 was tolerant in concentrations up to 2% of Atrazine in both

Table 2

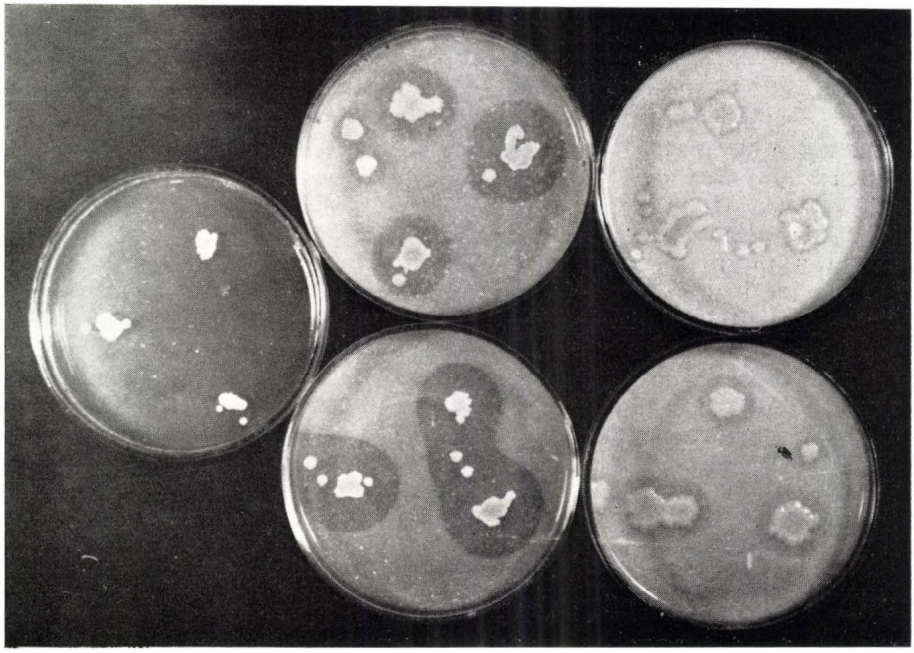
The tolerance of bacteria to herbicides

Number of bacteria	Control	2% Glucose			5% Glucose			2% Glucose			5% Glucose		
		0.1%	0.5%	2.0% A	0.1%	0.5%	2.0% A	0.1%	0.5%	2.0% P	0.1%	0.5%	2.0% P
2	1.05*	0.9	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.76	0.71	0.0	0.0	0.0	0.0	0.0	0.70	0.72	0.77	0.65	0.0	0.0
5 K	0.92	0.85	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	0.70	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	0.83	0.75	1.21	1.48	0.72	0.93	1.48	0.84	0.91	0.89	0.86	0.85	1.50
23	1.43	1.20	1.30	1.50	1.10	1.70	1.80	2.0	2.20	0.0	1.10	2.25	1.60

A = Atrazine
P = Prometryne
* = cm

variants, with 2% and 5% of added sugar. It should be emphasized that the tolerance and growth of bacteria is greater in higher concentrations of Atrazine, for both variants, compared with the growth and size of the control (without herbicides); while the average growth and size of colonies on control media was 0.83 cm the colonies on media with 2% Atrazine were 1.48 cm. The same strain showed tolerance to Prometryne too, especially in the variant with 5% sugar and 2% Prometryne (Figs 1 and 2).

Fig. 1. The growth of *Arthrobacter spp.* 11; Left: Control plate with 2% glucose only; plates above: left with 2% glucose and 0.5% Atrazine, rigth with 2% glucose and 2% Atrazine; plates below: left with 5% glucose and 0.5% Atrazine, right with 5% glucose and 2% Atrazine



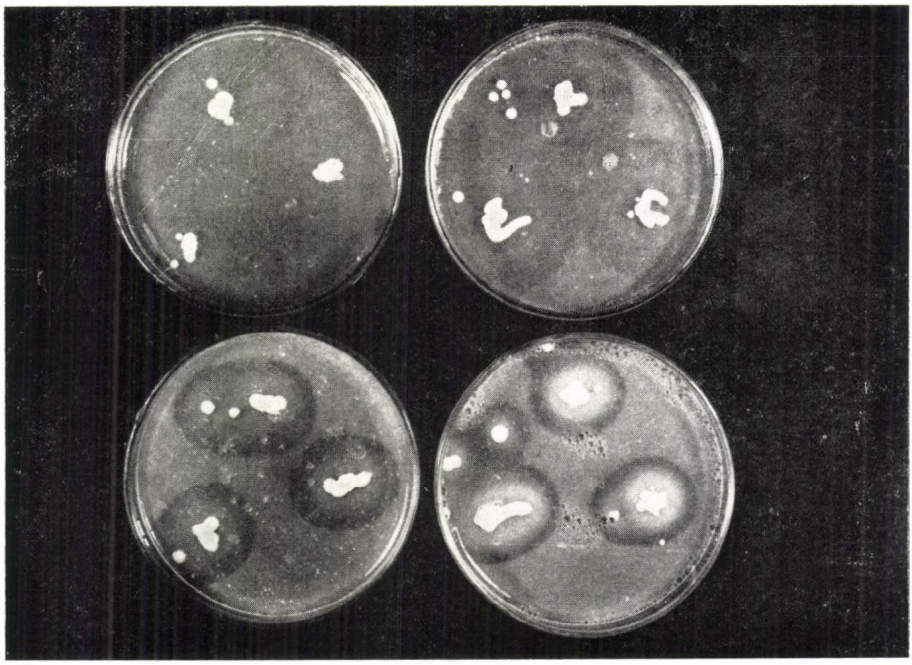


Fig. 2. The growth of *Arthrobacter spp.* 11; Above left control with 2% glucose only, rigth: with 5% glucose and 0.1% Prometryne; below left: with 5% glucose and 0.5% Prometryne, right: with 5% glucose and 2% Prometryne

Bacterium No. 23 also showed tolerance to herbicides compared with the control. The tolerance to Atrazine was evident in both variants; but it was more distinct with higher concentrations, so that the largest growth of colonies were on media with 2% Atrazine. The tolerance to Prometryne of this strain was expressed in both variants too, the largest growth and tolerance was found in concentration of 5% Prometryne in both variants. In variants with 5% sugar and 2% Prometryne a slight depression was noted, while in variants with 2% sugar and 2% Prometryne there was no growth i.e. herbicide inhibited. This demonstrated that the higher quantity of sugar (organic matter) decreases the depressive or toxic effect of the herbicide (Figs 3, 4).

These two distinct tolerant bacteria No. 11 and 23, were afterwards, examined individually on the possibility of utilizing the herbicides as sole source of carbon, and in this way of destroying them. The results in Table 3 show that strain 11 had a better growth in media with Atrazine and Prometryne, especially in higher concentrations, compared with the control, without herbicides. This may have happened with the utilization of carbon and degradation of herbicides. Strain 23 showed also growth on media with herbicides, but only poorly up to 1% of Prometryne, indicating a degradation of lower intensity. Higher concentration than 1% of Prometryne had a depressive influence which has occurred in the tolerance investigation too.

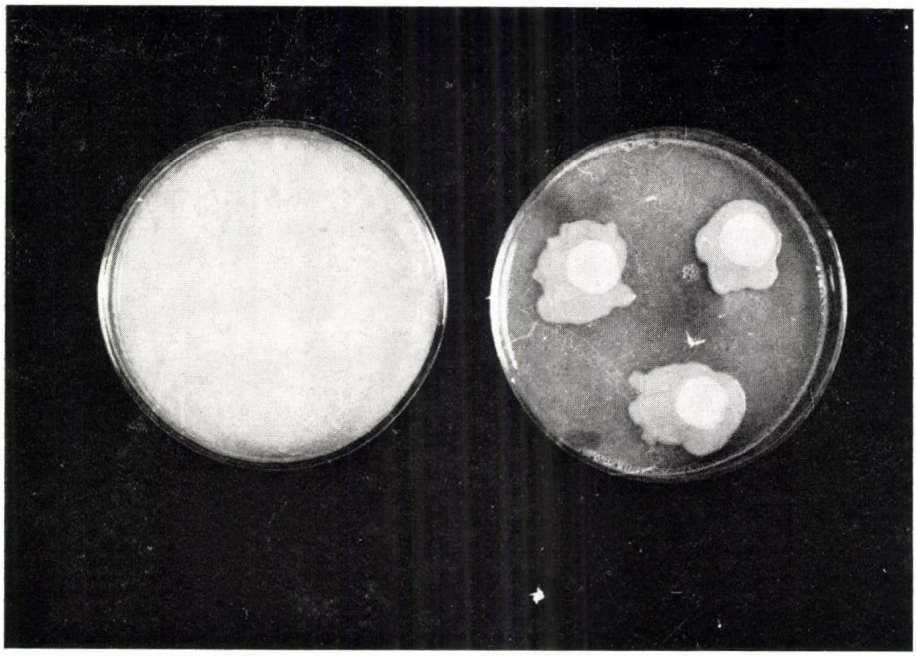


Fig. 3. The growth of *Arthrobacter spp.* 23; plate on left with 2% glucose and 0.5% Prometryne, plate on right with 2% glucose and 2% Prometryne

Table 3

The utilization of carbon from herbicides by bacteria

Number of bacteria	Control	Atrazine					Prometryne				
		0.1%	0.2%	0.5%	1.0%	2.0%	0.1%	0.2%	0.5%	1.0%	2.0%
11	0.83*	0.93		0.80		1.10	0.92		0.90		1.20
23	1.43	0.70	0.72	0.78	0.64	0.41	0.51	0.71	0.79	0.0	0.0

* = cm

The results confirmed the phenomenon that for the effect of herbicides on soil microflora, besides nature the concentration and mode of application of herbicides is also important. The utilization of herbicides as sole source of carbon, and their degradation by some bacteria, depends on their nature, concentration and the presence of organic matter, in this case of sugar, too (Grover 1966; McCormick and Hiltbold 1966).

SUMMARY

In field experiments on alluvial soil types the influence of Agelon (Atrazine and Prometryne) on soil microflora was investigated. In both sampling fungi were stimulated, while actinomycetes were depressed.

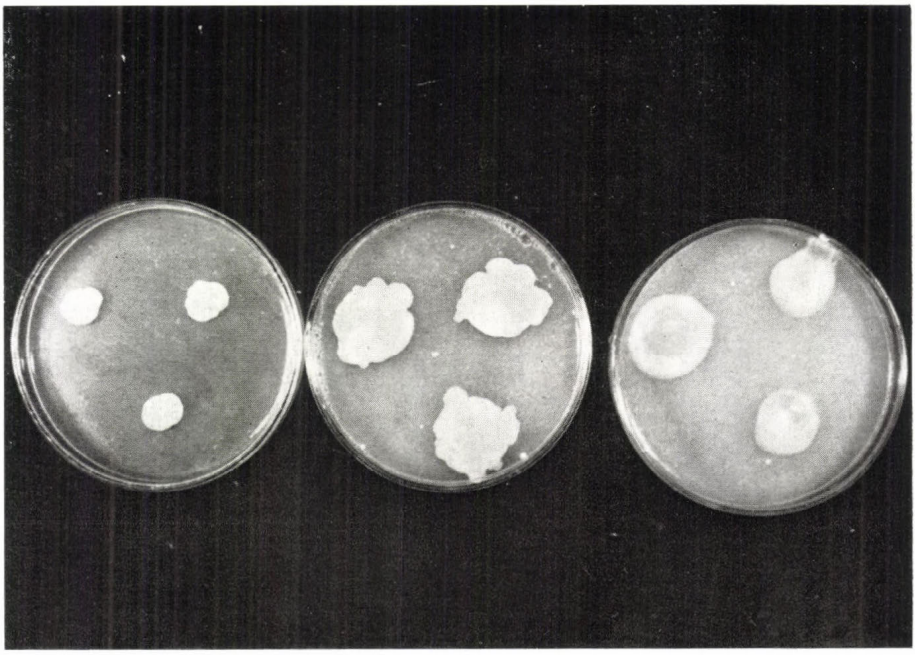


Fig. 4. The growth of *Arthrobacter spp.* 23; plates with 5% glucose and 0.1%, 0.5% and 2% Prometryne, from left to right

Individually the tolerance of some representatives of species of bacteria on Atrazine and Prometryne was examined as well as the possibility of using these herbicides as sole source of carbon, and in this way of destroying them. *Arthrobacter sp.* 11 showed tolerance to Atrazine and Prometryne in both variants — with 2% and 5% sugar added. This bacterium can degrade the herbicides, since it uses them as sole source of carbon.

Arthrobacter sp. 23 showed also tolerance to herbicides and degraded them utilizing as sole source of carbon. The tolerance and utilization was poorly expressed in Prometryne. The results confirmed the phenomenon that the greater quantity of sugar (organic material) support the lowering of depressive or toxic effects of herbicides.

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MICROBIOLOGICAL ACTIVITY AND BREAKDOWN OF "CASORON" HERBICIDE IN THE SOIL

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Many herbicides used in weed control come in contact with soil micro organisms. Owing to the great importance of microorganisms for soil fertility it is necessary to establish how they are affected by herbicides.

This study aimed to assess the microbiological effect and breakdown of Casoron in the soil.

METHOD

The effect of the Casoron herbicide on soil microorganisms was assessed in a field experiment and in a model one in the greenhouse under controlled conditions. Trials were conducted in 1969 in the experimental field of the Plant Protection Institute, Kostinbrod on chernozem-smolnitza soil type in a black currant *(Ribes nigrum L.* var. *Europ.)* plantation. The herbicide was applied in the form of granulated actual material, in the dose of 7.5 kg/ha active ingredient. Soil samples were obtained 2, 20, 30, 60, 90 and 150 days after treatments from soil layers to 0—4 and 4—8 cm respectively.

Microbiological studies were conducted by plating diluted soil suspension on agarized nutrient media. The following important soil microorganisms were counted: bacteria on meat peptone agar; actinomycetes — on starch-ammonium agar; microscopic fungi — on Czapek's medium. Moreover free ammonifying bacteria and that of the *Azotobacter* genus were studied.

Detoxication of Casoron was assessed by determining residual herbicides in the soil after a biological method, developed at the Plant Growing Institute, the Netherlands. The experiment was carried out in four replications in pots containing 300 g air dry soil in each 5 bean seeds *(Phaseolus vulgaris L.)* were sown. Plants were grown in the greenhouse at 65 of the water capacity and the temperature was 20—22° C. Fresh and dry weight of plants above cotyledons were determined 4 weeks after cultivation. Herbicide residues were calculated in gamma active ingredient /1000 g soil.

RESULTS

Total count of ammonifying bacteria decreased as affected by the herbicide used (Fig. 1). Field experiment data on the surface soil (0—4 cm) are particularly significant in this connection. Compared to the control bacterial count was 3 times lower in analyses 60 and 90 days after treatment. In the lower soil layer their count diminished as well. Both studies show a gradual

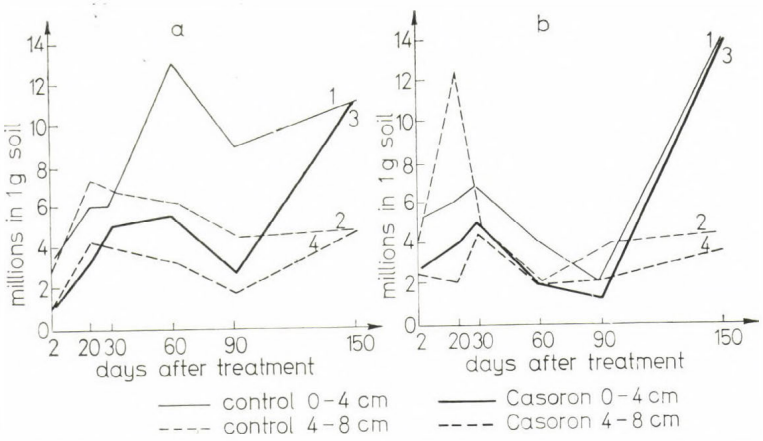
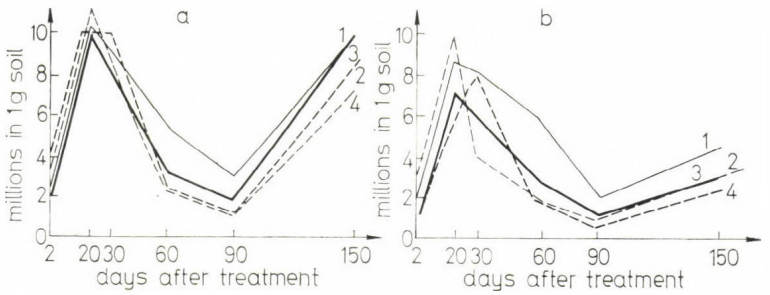


Fig. 1. Bacteria *a)* field experiment, *b)* greenhouse experiment. 1) control 0—4 cm; 2) control 4—8 cm; 3) Casoron 0—4 cm; 4) Casoron 4—8 cm

decreasing effect of the herbicide which ceases after the 90th day. Therefore analyses on the 150th day after treatment did not show any count differences of bacteria in the treated and control variants. According to findings, maximal toxicity of the herbicide to the bacteria become manifest only after it was allowed to stay in the soil for a longer period. Thus the inhibition level in the surface soil was highest 60 to 90 days after treatment. This phenomenon may be due to the fact that herbicide is eliminated from granules and toxic decomposition products develop. The inhibition activity of the herbicide is lower in the 4 to 8 cm soil layer, attributable to the rather small quantity of actual material available in the soil (Fig. 4). On the whole, certain differences in data on the model experiment correlate those on the field. Thus, e.g. breakdown of the herbicides was more rapid due to optimal temperature and moisture conditions at the place where experimental pots were allowed to stay (Fig. 4). Therefore the maximal toxic effect became manifest up to the 20th day after treatment and was more apparent in the lower layer (4—8 cm). Analyses on the 30th day after treatment and later, showed smaller differences of the total bacterial number between treated and control variants. This is

Fig. 2. Actinomycetes *a)* field experiment, *b)* greenhouse experiment



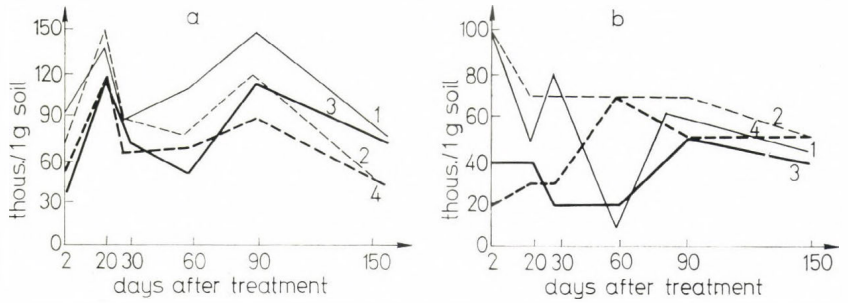


Fig. 3. Microscopic fungi *a)* field experiment, *b)* greenhouse experiment

attributable to the enhanced detoxication of the herbicide. Analyses carried out 150 days after treatment disclosed an insignificant if any inhibition effect. Casoron did not affect substantially nitrogen-fixing bacteria of the *Azotobacter* genus.

In field trials Casoron affected actinomycetes in a similar way as bacteria (Fig. 2). This group of microorganisms was also inhibited by the herbicide and as to bacterial number differences in total number, Actinomycetes were less important between treated and control variants. Besides the actual material had a poor if any effect on these microorganisms in the lower soil layer (4—8 cm). Certain morphological particularities of Actinomycetes, such as their rather dense cellular membrane, reducing the penetration effect of actual materials, may account for the poorer expression of the herbicidal effect.

On the other hand their enzyme system is stronger as a result of which they are less severely affected by environmental conditions (Mishustin 1956). On the whole, data on the model experiment were confirmed by the results of the field experiment. Up to a certain extent the latter more clearly expressed the inhibiting herbicidal effect in the surface layer (0—4 cm). On the 30th day after application analyses of the lower layer (4—8 cm) showed twice more actinomycetes in the treated variant. The stimulation effect most probably can be ascribed to the utilization of certain decomposition products of the herbicide, by the auxiliary enzyme system of these microorganisms.

The number of microscopic soil fungi was also inhibited by the herbicide (Fig. 3). In the field experiment higher toxic effect was noted in the surface soil layer (0—4 cm) on the 60th day after treatment. A certain effect was shown on the 90th day as well. Studies 150 days after treatment disclosed no particular

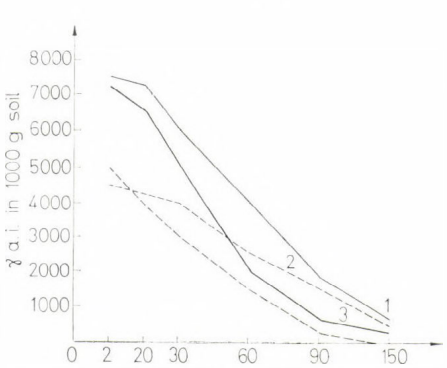


Fig. 4. Detoxication of Casoron in soil. 1) 0—4 cm field; 2) 4—8 cm field; 3) 0—4 cm greenhouse; 4) 4—8 cm greenhouse

number differences in treated and control plots as noted with the other microorganisms. Under existing conditions there are minimal herbicide quantities in soil 500—600 ng active material /1000 g soil (Fig. 4). According to analyses of the model experiment the count of fungi as affected by the herbicide decreased more intensively. Similar to other microorganisms the toxic effect became manifest up to the 30th day after treatment.

Experimental results on the decomposition of herbicide in the soil are presented in Fig. 4. In the model experiment, carried out under controlled conditions as compared to the field breakdown of the herbicide was relatively more rapid. The highest decomposition level was on the 60th day after applying herbicide to soil upon which intensity of the detoxication process gradually decreased.

DISCUSSION AND CONCLUSIONS

In connection with the analyses of the toxic effect of Casoron herbicide on ammonifying bacteria, Micev and Bubalov's studies (1969) on the same preparation used under wheat field trial are of interest. Analyses on its effect during the latter treatment stages showed stimulation of the bacterial number. This finding is not at variance with ours as the establishment of prerequisites for a rapid development of certain microorganisms in the biocenosis of the soil is possible under specific conditions when the inhibition effect has ceased. This is confirmed by Audus (1964) statements concerning the adaptation of certain microorganisms to an environment containing herbicides and existing possibilities for outyielding the remaining microorganisms of the cenospecies. In this case certain microorganisms probably become more competitive in respect to nutrients and can utilize herbicidal decomposition elements as nutrition sources. Thus studies definitely confirm the possibility of utilizing herbicides as nitrogen and carbon sources (Guillemat 1960).

Faster detoxication of herbicides was established in heavy soils as compared to sandy ones. Evaporation is an important factor for Dichlobenyl losses from soil. Residual Dichlobenyl was assessed 105 days after incorporation into the soil during a biologic experiment by Barnsley and Rosher (1961). This experiment showed 500—600 gamma of actual material/1000 g soil with the field experiment and 300—400 gamma respectively with the model one 150 days after Casoron treatments. Sheets, Harris and Smith (1969) found 0.56 ppm to be the lowest biologically determinable Dichlobenyl concentrations. Hence these studies confirm our findings. Generally the followings could be concluded:

1. Casoron herbicide applied in the dose of 7.5 kg/ha active ingredient on chernozem-smolnitza soil type inhibited the growth of soil bacteria, actinomycetes and microscopic fungi. The preparation had no effect on the nitrogen-fixing bacteria of the Azotobacter genus.
2. Under natural conditions the inhibition effect of the material was assessed up to the 90th day after treatment.
3. In the greenhouse under controlled conditions detoxication of Casoron in the soil was more rapid and slower in the field. In a field experiment residual Casoron was detected on the 150th day after application.

SUMMARY

The effect of the Casoron herbicide in a dose of 7.5 kg/ha active ingredient on soil microflora was studied in chernozem-smolnitza soil type in a field trial with black currants. At the same time model experiments under greenhouse conditions were conducted to check the effect of the preparation under controlled conditions. The herbicide inhibited the number of soil bacteria, actinomycetes and microscopic fungi tested.

Nitrogen-fixing bacteria of the Azotobacter genus were only insignificantly affected by the herbicide.

Under natural conditions the number of microorganisms studied was inhibited by the herbicide up to the 90th day after treatment.

Decomposition of casoron in soil was assessed after the biological method.

Residual herbicide was found in the soil on the 150th day after treatment.

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RELATIONSHIP BETWEEN DALAPON AND TRICHLOROACETIC ACID HERBICIDES AND THE MICROFLORA OF LIGHT BROWN SOILS

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The purpose of our work was to study the interrelationship between herbicides and the main physiological groups of soil microflora. Both laboratory as well as field experiments with Dalapon and trichloroacetic acid (TCA) have been conducted.

The laboratory tests were carried out with sterile and non-sterile soils. Herbicide preparations were added to soils in water solutions in the following concentrations: Dalapon — 1, 2, 12, 720 and TCA — 60, 600, 720 mg per 200 g of soil. Water was added to the soil samples up to 60% of their water-holding capacity and incubated under 27—28 °C in Koch dishes. The toxicity of herbicides was determined by biotests using wheat.

The data (Table 1) indicate that the degradation rate of herbicides proceeds more rapidly under the conditions of non-sterile than of sterile soils.

Table 1

Degradation of herbicides in the soil (laboratory tests)

Tested variants (herbicides in mg/g) of soil	Soil	Number of shoots in different dates							
		24/4	27/4	9/5	12/5	25/5	28/5	25/6	28/6
Control (without herbicide)	Sterile	8	22	12	25	21	25	25	25
	Non-sterile	10	25	18	25	15	25	23	25
Dalapon 1.2 mg/200 g	Sterile	0	0	0	0	3	8	23	25
	Non-sterile	0	0	3	7	17	21	21	25
Dalapon 12 mg/200 g	Sterile	0	0	0	0	6	8	19	25
	Non-sterile	0	0	2	6	13	19	25	25
Dalapon 720 mg/200 g	Sterile	0	0	0	0	0	0	0	0
	Non-sterile	0	0	0	0	0	0	0	0
STCA 6 mg/200 g	Sterile	0	0	0	0	10	12	21	25
	Non-sterile	0	2	7	11	21	25	20	25
STCA 60 mg/200 g	Sterile	0	0	0	0	7	10	18	25
	Non-sterile	0	0	3	8	19	23	22	25
STCA 720 mg/200 g	Sterile	0	0	0	0	0	0	11	13
	Non-sterile	0	0	0	0	7	10	20	24

Evidently it is due to the activity of soil microflora. Dalapon and sodium trichloroacetate (STCA) high concentrations are not completely degraded.

The degradation of herbicides in the field has been studied. The tests of these compounds obtained by paper chromatography have shown that herbicides are completely degraded during the vegetation of plants, Dalapon after 3.5 months, STCA — after 3 months.

The detoxication rate is dependent on the meteorological conditions as well as on the humidity and temperature.

The influence of various concentrations of Dalapon and STCA on different groups of soil microflora has been studied in light brown soils (Table 2).

Table 2

The influence of Dalapon on soil microflora in field tests (Number of microorganisms in million per g)

Media	Physiological groups	Tests	Days of observations					
			May 1	May 10	June 30	July 60	Sept. 90	Oct. 135
MPA	Total number of micro-organisms	Control	11.2	15.2	20.2	12.8	18.7	14.7
		Dalapon 5 kg/ha	10.0	12.5	8.8	8.7	11.7	18.7
		Dalapon 8 kg/ha	5.1	10.1	10.5	8.7	5.5	12.0
SAA	Total number of bacteria and actino-mycetes	Control	22.5	10.0	8.7	7.2	17.0	14.0
		Dalapon 5 kg/ha	3.0	10.2	12.2	10.0	10.0	10.0
		Dalapon 8 kg/ha	2.5	3.6	18.2	17.2	11.2	18.2
Ashby-agar	Oligonitro-phyles	Control	5.5	20.5	10.2	5.5	20.2	18.7
		Dalapon 5 kg/ha	5.0	20.6	9.7	9.7	11.0	17.5
		Dalapon 8 kg/ha	3.7	14.5	17.0	17.0	16.2	17.0
MPA + must	Spore-forming bacteria	Control	17.0	2.7	0.5	1.7	3.0	4.0
		Dalapon 5 kg/ha	13.6	3.0	0.3	1.0	1.7	1.7
		Dalapon 8 kg/ha	3.3	2.1	0.7	1.5	0.5	1.7
Peptone water	Ammonifiers	Control	25.0	25.0	25.0	25.0	25.0	6.0
		Dalapon 5 kg/ha	25.0	6.0	6.0	6.0	25.0	6.0
		Dalapon 8 kg/ha	6.0	6.0	6.0	6.0	25.0	6.0

The results of investigations indicate that the tested herbicides have different effects on soil microorganisms. Dalapon inhibits almost all groups of soil microflora during 60 days after its addition into the soil. Upon which the inhibitory action of Dalapon disappears and the increase of the growth of microflora is observed, indicated by plate counts of microorganisms, grown on starch-ammonium agar medium (SAA). The increase in numbers of microorganisms may be due to the adaptation of microflora to Dalapon or its degradation products. Spore-forming bacteria are more sensitive to Dalapon rather than non-sporeformers. This herbicide has no high inhibitory action on the growth of nitrifying ammonifying and cellulose decomposing microorganisms. The inhibition of *Azotobacter* which is so distributed in the investigated soils has not been observed (Table 3).

Table 3

Development of different groups of soil bacteria after the addition of Dalapon to the soil
(Number of microbial overgrowth of 100 g of lumps in per cent)

Media	Microorganisms	Tests	Days of observations					
			May 1	May 10	June 30	July 60	Sept. 90	Oct. 135
Ashby agar	Azotobacter	Control	100	100	100	100	100	100
		Dalapon 5 kg/ha	100	100	100	100	100	100
		Dalapon 8 kg/ha	100	100	100	100	100	100
Hutchinson's agar	Cellulose decomposing micro-organisms	Control	31	49	78	57	62	97
		Dalapon 5 kg/ha	39	38	59	37	53	91
		Dalapon 8 kg/ha	34	40	59	58	60	93
Winogradsky's medium	Nitrifiers	Control	35	73	73	57	78	82
		Dalapon 5 kg/ha	41	68	53	68	72	72
		Dalapon 8 kg/ha	57	68	72	82	72	73

The mixing of STCA into the soil during the first 10 days has a stimulatory action on almost all physiological groups of microorganisms. After 30—60 days the degradation of this herbicide takes place and inhibition is observed. STCA has an inhibitory action on the growth

Table 4

Influence of STCA on soil microflora
(Field tests, number of microorganisms in million per g of soil)

Media	Microorganisms	Tests	Days of observations					
			May 1	May 10	June 30	July 60	Sept. 90	Oct. 135
MPA	Total number of micro-organisms	Control	14.1	6.0	24.3	15.6	22.5	17.7
		STCA 20 kg/ha	29.4	13.2	6.0	4.0	11.4	16.1
		STCA 40 kg/ha	37.2	10.0	6.0	4.6	12.0	16.6
SAA	Total number of bacteria and actino-mycetes	Control	3.3	11.4	10.5	28.5	21.0	16.6
		STCA 20 kg/ha	10.2	18.3	9.6	24.0	12.8	18.9
		STCA 40 kg/ha	28.2	16.3	4.9	10.6	16.5	17.2
Ashby agar	Oligonitro-phyles	Control	7.5	17.4	12.3	21.6	24.3	22.5
		STCA 20 kg/ha	9.3	32.7	6.3	11.7	21.0	24.0
		STCA 40/kg/ha	10.2	32.1	7.6	16.5	18.0	37.6
MPA+must	Spore-forming bacteria	Control	6.0	0.3	0.6	0.3	4.5	4.8
		STCA 20 kg/ha	7.2	0.9	0.9	0.9	2.1	2.7
		STCA 40 kg/ha	7.2	1.0	0.7	0.8	2.1	2.7
Peptone water	Ammonifiers	Control	6.0	6.0	13.0	25.0	6.0	2.5
		STCA 20 kg/ha	7.0	25.0	25.0	6.0	2.5	2.5
		STCA 40 kg/ha	25.0	25.0	25.0	6.0	2.5	2.5

of microorganisms grown on SAA, their number decreasing 2—2.5 times, and the number of microflora grown on MPA (meat-peptone agar) 4 different sampling times (Table 4).

In laboratory and field tests the treatment of soils with herbicides has no inhibitory influence on the development of soil microorganisms. The addition of doses used in practice of STCA to the soil stimulates the growth of microorganisms grown on MPA (Table 5). The increase of the herbicide concentration up to 400 mg/kg decreases the number of soil microorganisms. For example, in experiments with the use of STCA in concentration 40 mg/kg was 6.9 million of microorganisms per/g of soil but in concentration 400 mg/kg their number was about 3 million cells/g. The inhibitory action of STCA was observed up to 40 days after which it disappeared.

Table 5

Influence of STCA on soil microflora
(Field tests, number of microorganisms in millions per/g of soil)

Media	Microorganisms	Tests	Days of observations		
			1	20	40
MPA	Total number of microorganisms	Control	4.9	2.7	3.6
		STCA 40 mg/kg	6.5	3.9	3.3
		STCA 400 mg/kg	3.1	2.0	2.2
Ashby agar	Oligonitrophyles	Control	3.9	3.3	1.5
		STCA 40 mg/kg	4.1	4.2	4.5
		STCA 400 mg/kg	3.1	4.6	4.5
SAA	Total number of bacteria and streptomycetes	Control	4.9	3.2	4.5
		STCA 40 mg/kg	3.0	2.3	3.4
		STCA 400 mg/kg	5.7	5.8	2.7
MPA+must	Spore-forming bacteria	Control	0.2	0.05	0.1
		STCA 40 mg/kg	0.2	0.07	0.05
		STCA 400 mg/kg	0.2	0.01	0.05
Peptone water	Ammonifiers	Control	2.5	2.5	2.5
		STCA 40 mg/kg	1.3	0.25	2.5
		STCA 400 mg/kg	0.25	0.6	2.5

The stimulatory action of STCA on the oligonitrophyle bacteria and *Pseudomonas* has been observed. This herbicide has no valuable influence on the development of spore-forming bacteria and actinomycetes, but in some cases stimulates the growth of *Bacillus cereus*, *Bacillus mesentericus*.

Dalapon has a different effect on some physiological groups of microorganisms. In laboratory experiments it displays weak inhibitory action on the total number of microorganisms and strong inhibition on *Actinomycetes*. Spore-forming bacteria and fungi are also inhibited, but after 20 days this action disappears (Table 6).

Dalapon as well as STCA stimulate the development of oligonitrophyle bacteria and *Azotobacter*.

Table 6

Influence of Dalapon on the microflora of light brown soils (Laboratory tests, microorganisms in millions per g)

Media	Microorganisms	Tests	Days of observation		
			1	20	40
MPA	Total number of microrganisms	Control	4.9	2.7	3.6
		Dalapon 8 mg/kg	3.5	2.5	3.9
		Dalapon 80 mg/kg	3.5	2.7	2.2
Ashby agar	Oligonitrophyles	Control	3.9	3.3	2.5
		Dalapon 8 mg/kg	4.5	3.3	3.7
		Dalapon 80 mg/kg	7.7	4.6	2.6
SAA	Total number of bacteria and actinomycetes	Control	4.9	4.2	4.4
		Dalapon 8 mg/kg	3.8	3.5	2.6
		Dalapon 80 mg/kg	3.0	2.8	3.5
MPA+must	Spore-forming bacteria	Control	0.2	0.05	0.1
		Dalapon 8 mg/kg	0.1	0.2	0.1
		Dalapon 80 mg/kg	0.1	0.2	0.1
Peptone water	Ammonifiers	Control	2.5	2.5	2.5
		Dalapon 8 mg/kg	11.1	6.0	2.5
		Dalapon 80 mg/kg	11.1	7.0	2.5

The data have shown that herbicides which do not decrease the total number of microorganisms have a specific effect on different groups of soil microflora.

Our investigations with pure cultures have indicated that Dalapon and STCA in concentrations of 8—40—80—400 mg/ml reveal different effects on different species and sometimes strains of the same species of microorganisms. In general, Dalapon is more toxic than STCA. For example, Dalapon inhibits all tested cultures of *Actinomycetes* but STCA has no action. Tested strains of nodule bacteria were more resistant to STCA.

The above-mentioned data indicate that the herbicides tested in practical doses have no essential and prolonged effect on soil microflora.

SUMMARY

Investigations were conducted to clarify how the soil microbiological processes were influenced by the herbicides Dalapon and Na-trichloroacetate.

It was established that the inactivation of the examined herbicides took place much more slowly in sterile soils than in septic conditions.

The results of field experiments proved that Dalapon in 3.5 months and the Na-trichloroacetate in 3 months broke down in the soil. The detoxication strongly depends on the moisture conditions.

Dalapon and Na-trichloroacetate significantly influenced the related ratio of microorganisms belonging to several physiological groups.

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RELATIONSHIP BETWEEN HERBICIDE DYMID AND SOIL MICROORGANISMS

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Dymid (N,N-dimethyl-2, 2-diphenylacetatamide) is a new herbicide, produced by Elanco Product Company. This herbicide has a wide action spectrum against different weeds. It is used for protection of the seedlings of some vegetables, and especially for tobacco.

As regards the influence of Dymid on the soil microflora, there are very little data available. According to some data provided by Elanco Company, the degradation and destruction of Dymid in the soil is very slow.

MATERIAL AND METHODS

During the examination of this relationship we have used two types of soils: red soil, with neutral reaction, and alluvium, also with neutral reaction. Both soil types are poor in humus.

The soil samples received doses of Dymid equivalent to that used in practice namely 6 and 8 kgs per hectar (as a first and second concentration). The preparation had 80% active ingredient. The moisture of the soil was adjusted to about 60% of soil moisture capacity. The herbicide was mixed with the soil in the Petri dishes containing 300 g from each soil type. Dishes were incubated at room temperature, 22–25 °C. Microbiological analyses were made after 10 and 60 days from the addition of the Dymid to the soil.

We examined the influence of Dymid upon the asporogenic bacteria, bacterial spores, fungi as well as *Actinomycetes*, using the plate count method. For the bacteria, broth agar was used, for the bacterial spores the same agar, warmed up at a temperature of 80 °C for 10 minutes after the inoculation, while for the fungi we used the Čzapek-Dox agar, with 0.01% of streptomycin sulphate, and for the *Actinomycetes* Waksman's agar.

Then the influence of Dymid on *Azotobacter*, on the nitrifying bacteria as well as on the cellulolytic microorganisms was investigated. The Winogradsky's silicagel as a nutrient medium was used.

When studying the ability of microorganisms to use Dymid as a sole source of nitrogen or carbon, we used Jensen's agar, and the nitrogen or carbon component replaced by 0.1% Dymid.

RESULTS AND DISCUSSION

a) *Influence of Dymid on soil microflora.* The results obtained regarding the influence of Dymid on heterotrophic soil microflora are shown in Table 1. These results indicate that the first, and especially the second concentration of Dymid has a negative influence on the examined groups of soil microorganisms ten days after the addition of Dymid to the soil.

Table 1

Influence of Dymid on heterotrophic microorganisms, per gram of absolutely dry soil

Dymid treatments	Asporogenic bacteria	Decreasing %	Bacterial spores	Decreasing %	Fungi	Decreasing %	Actinomycetes	Decreasing %
Red soil, 10 days after the introduction of Dymid into the soil								
Control	1,500,000	100.0	45,000	100.0	27,000	100.0	360,000	100.0
I conc.	1,100,000	26.7	38,000	15.6	13,300	50.8	300,000	15.0
II conc.	800,000	44.7	25,000	44.5	10,000	65.6	260,000	28.0
Alluvium, 10 days after introduction of Dymid into the soil								
Control	20,000,000	100.0	1,340,000	100.0	165,000	100.0	9,000,000	100.0
I conc.	15,000,000	25.0	1,200,000	11.2	76,000	53.8	8,400,000	0.7
II conc.	13,000,000	35.0	1,000,000	26.2	60,000	63.9	7,200,000	20.0
Red soil, two months after introduction of Dymid into the soil								
Control	2,100,000	100.0	37,000	100.0	32,000	100.0	470,000	100.0
I conc.	1,800,000	14.3	33,000	10.8	17,000	46.9	380,000	19.1
II conc.	1,000,000	52.4	25,000	32.9	12,000	62.5	300,000	36.2
Alluvium, two months after introduction of Dymid into the soil								
Control	23,700,000	100.0	730,000	100.0	210,000	100.0	8,750,000	100.0
I conc.	19,300,000	19.0	660,000	10.0	170,000	19.1	8,150,000	0.9
II conc.	13,700,000	42.2	560,000	23.2	130,000	38.1	7,000,000	20.0

After two months there was still a negative influence of Dymid on the examined groups of soil microorganisms. The decreasing % was somewhat smaller than in the case of 10 days after introduction of Dymid into the soil. The highest negative influence was found in the case of the asporogenic bacteria and the fungi in both soil samples.

The results obtained from the investigations of the influence of Dymid on some physiological groups of soil microflora, are shown in Table 2.

After 10 days from the addition of Dymid to the soil, negative influence was shown in both soil types on the examined physiological groups of microorganisms, with both applied concentrations. The smallest negative influence was recorded with *Azotobacter*, and the highest one with nitrifying bacteria.

After two months there was a negative influence with *Azotobacter*. With nitrifying bacteria and cellulolytic microorganisms, Dymid still showed negative

Table 2

Influence of Dymid on the number of some physiological groups of microorganisms

Treatment	Azotobacter % of fertile grains	Soil plaques	Nitrifying bacteria % of fertile grains	Cellulolytic microorganisms fertile grains			
				Total	Fungi	Bacteria	Actino mycetes
Red soil, 10 days after introduction of Dymid into the soil							
Control	80.8	+++	21.7	88.6	40.2	41.2	7.2
I conc.	73.6	+++	11.8	82.8	42.6	36.2	4.0
II conc.	72.2	++	0.0	60.5	34.3	24.0	2.2
Alluvium, 10 days after introduction of Dymid into the soil							
Control	82.7	++++	29.3	56.3	7.0	36.3	18.0
I conc.	79.7	++++	10.3	47.7	4.7	22.0	21.0
II conc.	74.2	+++	7.7	36.0	2.3	16.0	17.7
Red soil, two months after introduction of Dymid into the soil							
Control	81.3	+++	24.0	90.3	40.0	44.0	12.3
I conc.	80.0	+++	13.3	87.0	35.7	39.0	12.3
II conc.	78.7	+++	4.3	75.7	32.7	34.0	9.0
Alluvium, two months after introduction of Dymid into the soil							
Control	100.0	++++	31.7	85.0	9.7	55.3	20.0
I conc.	100.0	++++	20.3	75.3	9.0	49.3	17.0
II conc..	96.3	++++	8.3	64.7	8.7	40.3	15.7

influence. In all cases the negative influence was higher with the second concentration of Dymid.

b) *Influence of microorganisms on Dymid.* Like some other herbicides, Dymid can be used as a sole source of nitrogen or carbon for microorganisms. These results are shown in Table 3.

Very many isolates can grow on Dymid as a sole source of nitrogen, better than on Dymid as a sole source of carbon. The growth was very poor on Dymid as a source of carbon.

Some of these microorganisms can fix small quantities of nitrogen from the air as the oligonitrophyl microorganisms. We have done some experiments using Ashby nitrogen-free agar. Only the *Azotobacter*, the *Agrobacterium radiobacter* and the *B. polymixa* can grow on this medium. Other microorganisms utilized the nitrogen of Dymid.

SUMMARY

The interaction of Dymid and soil microorganisms was examined. From the results we concluded the following:

1. Dymid, in equivalents of practical field doses (6 and 8 kgs per hectar) depressed the number of the examined groups of heterotrophic soil microflora in two types of soils: neutral red and neutral alluvium.

The highest depression caused by Dymid was found in the case of the asporogenic bacteria and fungi. These results have been obtained within ten days after the introduction of Dymid into the soil.

Table 3

Growth in some microorganisms on the media with Dymid as a sole source of nitrogen or carbon

Microorganisms	Dymid as a sole source of nitrogen	Dymid as a sole source of carbon
Azotobacter chroococcum	++	+
Agrobacterium radiobacter	+	+ —
Bacillus megatherium	+	+
Bacillus mesentericus	+	—
Bacillus polymyxa	+	+ —
Pseudomonas fluorescens	+	+ —
Streptomyces griseus	+	—
Actinomyces candidus	+ +	+ —
Stachybotris atra	+ +	—
Pullularia pullulans	+	—
Cladosporium sp.	+ + +	+ —
Penicillium frequentans	+ +	—
Penicillium citreo-viride	+ + +	+
Fusarium sp.	+	+ —
Aspergillus terreus	+ +	+ —
Aspergillus niger	+ +	—
Alternaria tenuis	+ + +	—
Rhizopus nigricans	+	—
Trichoderma viride	+	—

+++ = very good growth
++ = good growth
+ = poor growth
+— = very poor growth
— = no growth

2. After two months there was still a negative influence of Dymid upon all the examined groups of microorganisms. This negative influence was somewhat smaller than ten days after the introduction of Dymid into the soil.

3. With regard to the physiological groups, after ten days there was a negative influence with all the examined groups, but the *Azotobacter sp.* was more resistant.

After two months there was still a negative influence with the nitrifying bacteria and with the cellulolytic microorganisms, but not with the *Azotobacter sp.*

4. There were some microorganisms which could grow on the media with Dymid as a sole source of nitrogen but only a few microorganisms, could grow very weekly, using Dymid as a sole source of Carbon.

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THE EFFECT OF GRAMOXONE ON N-FIXING MICROORGANISMS

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Nowadays chemicals have been used not only in agriculture in the course of so-called "chemical protection" against plant and animal pests but also, instead of mechanical weed control, chemical weed control has been applied more and more in the agriculture.

The pesticides introduced into the soil can influence the soil microorganisms. For this reason we studied whether the Gramoxone herbicide had an effect on the *Azotobacter* and *Rhizobium*.

MATERIAL AND METHODS

The growth of four strains of *Azotobacter chroococcum* was investigated on such a N-free solid medium of which 10^{-6} mg – 10^{4} mg Gramoxone was added to each litre in tenfold dilution steps. The composition of the medium was the following:

15.0 g mannitol	0.1 g $FeSO_4$
1.0 g K_2HPO_4	0.1 g $AlCl_3$
0.2 g $MgCl_2$	0.01 g $MnSO_4$
0.5 g $CaCO_3$	0.01 g $ZnSO_4$
0.2 g NaCl	2% agar-agar

1000 ml distilled water, pH = 7.2 (before sterilization). Sterilization at ½ atm. for 20 minutes.

Bacterial cultures grown for 48 hours on the same medium were streaked with loop onto the surface of the plate in two lines at a distance of 20 mm from each other. Four Petri dishes were used for all treatments and they were incubated at 28 °C.

The effect of Gramoxone was examined on 20 symbiotic N-fixing bacterial strains in vitro with dilution and agar gel diffusion method.

In the course of the test of the 11 different concentrations of the herbicide, smaller (1/100) and manifold (ten times) doses than used in agricultural practice were applied.

On this way 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.75 mg Gramoxone was added into 1000 ml medium. Such appropriate dilution series were prepared so that one of them added to the Czapek's medium would have the required concentration. In the case of agar gel-diffusion method the appropriate dilutions were applied into the holes made into the agar-agar. In this case the bacteria were suspended

in a thin agar layer which was poured to the surface of the plates. In the dilution method the microorganisms were suspended into the liquid medium with Takátsy's loop.

Further on vegetation experiments were carried out with Gramoxone to study whether the herbicide amount used in agricultural practice and 1/100 part of it had any influence on the rhizobial inoculation of pea.

The experiment was conducted in N-free Crone's nutrient solution using 1 litre glasses containing, 700 g washed quartz sand, quadruplication was carried out. The glass-pots were sterilized at 2 atm. pressure for 1 hour. The surface of Petit provance variety pea seeds were sterilized with 3% Chlorogenium solution. After they were washed with sterile distilled water five times. In every pot, five seeds were sown and they were inoculated with 0.2 ml bacterial suspension prepared according to Brown scale No 6 degree. One ineffective (No Bu 71) four effective (No Bu 9/6, Bu 9/7, Bo 9/3, Bo 9/4) *Rhizobium* strains were used for inoculation.

RESULTS AND CONCLUSIONS

The evaluation of the plates inoculated with *Azotobacter* was done on the basis of the growth compared to the control after two and five days (Table 1).

Table 1

Effect of Gramoxone on the growth of Azotobacter strains (Herbicide content mg/l medium)

Bacterial strains	Inhibition versus control		Total inhibition	
	After 2 days	After 5 days	After 2 days	After 5 days
1	10^{-6}	10^{-1}	1	10
2	10^{-6}	10^{-6}	10^{-1}	1
3	10^{-6}	10^{-6}	10^{-1}	10^{-1}
4	10^{-6}	10^{-5}	1	1

Two kinds of inhibition were differentiated: a smaller inhibition, when the growth compared with the control was only weaker and absolute inhibition, when there was no growth at all.

It could be proved after two days that the smallest 10^{-6} mg/l Gramoxone concentration applied, inhibited all the strains to a small extent. Complete inhibitions were recorded in the case of No. 1 and No. 4 strains at 1 mg dose and 10^{-1} mg/l herbicide doses inhibited absolutely the development of No. 2 and No. 3 strains. After five days smaller inhibitory effects in the case of No. 1 strain 10^{-1} mg/l, No. 2 and No. 3 strains 10^{-6} mg/l and No. 4 strain 10^{-5} mg/l doses were detected. Absolute inhibition was noticed in No. 3 strain at 10^{-1} mg/l, No. 2 and No. 4 strains at 1 mg/l and in the case of No. 1 strain at 10 mg/l herbicide doses. The most resistant was the No. 1 strain while the other proved to be sensitive.

Special attention should be paid to the fact that in all concentrations along the streak of No. 2 strain, isolated colonies did not developed continuous cultures as on the control plates. It demonstrated that in the population of No. 2 strain the variants were predominantly very sensitive to Gramoxone.

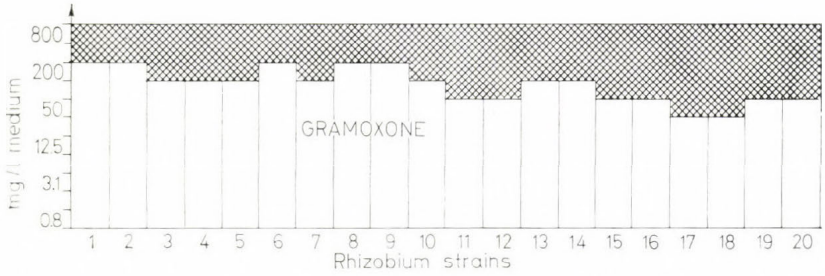

Fig. 1. The effect of Gramoxone on 20 different *Rhizobium* strains. (The shadowed part shows the inhibition rate.) 1—2 *Rhizobium* strains from *Glycine soja*, 3—7 *Rhizobium* strains from *Medicago sativa*, 8—14 *Rhizobium* strains from *Trifolium pratense*, 15—17 *Rhizobium* strains from *Pisum sativum*, 18—20 *Rhizobium* strains from *Vicia villosa*

From among the *Rhizobium* strains three originated from vetch *(Vicia villosa)* three from pea *(Pisum sativum)* seven from clover *(Trifolium pratense)* five from lucerne *(Medicago sativa)* two from soybean *(Glycine soja)* root nodules.

The effectiveness (root nodule formation and N-fixing capacity) of *Rhizobium* strains was previously checked on the basis of sterile pot experiments.

The results received with dilution and agar gel-diffusion method were not identical, the dilution method resulted inhibitory effect in more cases, thus it proved more sensitive to the to rhizobia than to the agar gel diffusion technique. For this reason the result obtained with the method will be reported (Fig. 1).

According to the investigations the dose of Gramoxone used in practice inhibited the growth of 40% of the strains.

The most sensitive rhizobia to Gramoxone proved to be the strains isolated from pea-vetch root nodules.

In the course of the vegetation experiment the Gramoxone treatments were conducted between sowing and *Rhizobium* inoculation. The experiment was evaluated six weeks after sowing on the basis of pod, stalk and leaf yield as well as the nodule number (Table 2). It could be established from

Table 2

Effect of Gramoxone on the growth of Pisum sativum

Rhizobium strains	Pods yield %			Stem + leaf %			Number of nodules %		
	Rh	Rh+G_1	Rh+G_2	Rh	Rh+G_1	Rh+G_2	Rh	Rh+G_1	Rh+G_2
Bü 71	100	31.9	93.3	100	68.8	84.6	0	0	0
Bü 9/6	100	43.0	95.5	100	72.3	100.7	100	31.1	70.0
Bü 9/7	100	48.0	113.2	100	68.4	107.5	100	39.3	157.1
Bo 9/3	100	55.3	87.1	100	84.3	88.0	100	71.3	71.3
Bo 9/4	100	66.5	118.2	100	88.6	125.8	100	67.7	171.7

Rh = Rhizobium inoculation
G_1 = 16 mg Gramoxone/pot
G_2 = 0.16 mg Gramoxone/pot

these, that the Gramoxone dose applied in practice (Gl) had a depressive effect on the yield and nodule number of all the plants inoculated with *Rhizobium* strains compared with the control. At the same time hundred-fold diluted concentration of the dose applied in practice had a similar effect to the control but in some cases-mainly as regards the number of nodules it had a stimulatory effect.

Thanks are due to Dr. J. Szegi for making the *Azotobacter chroococcum* strains available.

SUMMARY

The effect of Gramoxone on free-living aerobic nitrogen-fixing bacteria as well as on nitrogen-fixing bacteria symbiotic with leguminous plants was investigated. The growth of four strains of *Azotobacter chroococcum* on N-free culture medium with addition of 0.000 001 mg/l to 10,000 mg/l of Gramoxone was studied. The plates were incubated at 28 °C for 2 to 5 days and the growth was observed. After two days the growth of all strains studied was inhibited by dose of as little as 0.000 001 mg Gramoxone per litre. After five days only one strain behaved differently, all the other strains showed the same effect as after 48 hours.

The effectiveness (nodulating and nitrogen-fixing capacity) of 20 strains belonging to the species of *Rhizobium trifolii*, *Rh. meliloti*, and *Rh. japonicum* was controlled by means of pot cultures. The dilution method and the method of agar gel diffusion were used in the experiments. The result obtained by the two methods were not the same; the dilution method proved to be more sensitive — it revealed inhibitory effects in more cases — than the agar gel diffusion method. 40 % of the strains studied were inhibited by the usually applied doses of Gramoxone as indicated by the experiments. Rhizobia isolated from root nodules of pea-vetch were found to be the most sensitive to Gramoxone.

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A SURVEY OF HERBICIDE SENSITIVITY AND RESISTANCE OF RHIZOBIA

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Having dealt with the effect of fungicides, herbicides and insecticides on rhizobia since 1964, we could, in many cases compare our results obtained in Australia and Hungary, in laboratory, light room, glasshouse and field experiments. On the basis of the data, we could establish that there were pesticides which did not inhibit rhizobial growth in laboratory conditions, but which proved harmful to rhizobia and rhizobial inoculation in glasshouse and field. (Kecskés, 1970). In contrast, we found pesticides which resulted a similar negative effect on rhizobia under different environments (like Ceresan in Australia) or we received the same effect with Kloben, Aresin etc. herbicides in the field as in the laboratory (Borbély and Kecskés, 1970).

As regards the effect of herbicides on rhizobia, many articles have appeared (e.g. Vintikova et al. 1963 and 1964, Kaszubiak 1966, Avrov 1966, Garcia and Jordan 1967, Mikhaylova 1968, Avrov et al. 1968) dealing with the interaction of some herbicides or some rhizobial strains.

Due to the rapid development of herbicide research and its application in practice, the problem of rhizobial sensitivity and resistance to these herbicides forced us to do a general survey on this question. For this reason we studied the effect of 104 herbicides under laboratory conditions on 26 representatives of the six species of *Rhizobium* genus, hoping to receive some preliminary information — as regards the sensitivity or resistance of rhizobia — for our further work with these herbicides and rhizobia.

MATERIAL AND METHOD

The investigated strains of the different species of *Rhizobium* genus (according to Bergey's Manual) were chosen in such a way that they originated from the root nodules of different legume host plants grown under various climatic and other conditions of six countries from three continents and of different age, effectiveness, etc. Moreover, most of them were used for inocula of the different legume host plants cultivated in agricultural practice.

We wished to ensure in this way a generalization of our results as far as possible (Table 1).

The 104 herbicides were chosen from the same point of view mentioned above, so as to represent many chemical groups (Fryer and Evans 1968, Ubrizsy 1968) — sometimes many agents having the same active ingredients — prepared by companies of numerous countries for the control of different kinds of plants (Table 2).

As regards the investigation method — according to our aim and because of the tremendous number of tests — we chose a quite simple one (measuring the inhibitory zone) which was used by us in our earlier work (Kecskés and Vincent 1969). For this, saturated paper discs 7 mm in diameter triplicates herbicides in excess (from 0.49 cg/disc) were used with large Petri dishes (140 mm in diameter) containing 35 ml YMA medium.

Table 1
Rhizobium strains

Origin	Species and laboratory marking		
	Leguminosarum	*Phaseoli*	*Trifolii*
Australia	1) SU 391		9) WA 67
			10) WU 290
Bulgaria	2) 13/B		11) tri 383
	3) 22/B		
Czechoslovakia		6) D 407	
Denmark			
Hungary	4) Bo 8/1	1) Bab 8/7	12) Lo 133/64
	5) Bo 8/6		13) Lo 8/6
Poland		8) Fas 65	

Origin	Species and laboratory marking		
	Lupini	*Japonicum*	*Meliloti*
Australia			20) SU 47
Bulgaria		15) 76/D	21) mel. 7
		16) 34	22) mel. 200
Czechoslovakia			23) D 66
Denmark		17) 633	24) 56
Hungary		18) Soya 1	25) Lu 8/10
	14) Csf 8/3	19) Soya 3	
Poland			26) N 96

* SU 391: originally from USA
altogether: 26 strains
No. 1—26 = The number of strains listed in Table 2 (e.g. No. 1 = SU 391 etc.).

RESULTS

It would take a long time to discuss in detail all the data obtained during our investigations, therefore we should like to give a general review on the basis of the inhibitory rate of herbicides (Table 2), and the list of non-inhibitory herbicides (Table 3) as well as the herbicides having the strongest inhibitory effect (Table 4).

As it was demonstrated in Table 2 the stimulatory effect of herbicides was noted only in 14 cases of six strains of 5 species (No. 5, 7, 11, 18, 20,

22) but at the same time the No. 5 strain of *Rh. leguminosarum* and No. 7 strain of *Rh. phaseoli* had no inhibition at all — apart from strains No. 23 and No. 1, 24 — by the relatively greater number of herbicides. In contrast to this the No. 9 strain of *Rh. trifolii* was strongly inhibited by the greatest (76) number of herbicides.

The data of Table 3 demonstrate, that Venzar and Dachtal did not inhibit the growth of 50% of the observed strains, Aquathol G, Embutor E 64.6% and Planavin 68.4%. The majority of the strains (more than half) were strongly inhibited by Basamid, Balan, Avadex, Bexone, Aretit, Atracil C and though in this case the herbicides cannot be sharply differentiated according to chemical groups, nevertheless, thiocarbamates proved to be more inhibitory.

The comparison of the individual strains as the representatives (together) of the different species serve as interesting data.

As an end result it could be established that about half of the herbicides did not prove to be toxic to all 26 strains.

Thanks are due to Prof. J. M. Vincent, Dr. L. Raicheva, Dr. E. Hamatova, Prof. H. L. Jensen, Dr. W. Malisevska, Mrs. É. Bakondi and Mrs. B. Ocsay, moreover to my assistants Miss K. Schmidt and Miss Zs. Gebauer.

SUMMARY

The effect of 104 herbicides belonging to 26 different chemical groups was studied on 26 strains representing six species of *Rhizobium* genus in laboratory conditions.

The sensitivity and resistance of rhizobia to the investigated herbicides was noted on the basis of the inhibitory rate of herbicides.

The non-inhibitory herbicides and the herbicides having the strongest inhibitory effect were listed.

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Table 2

Inhibitory and stimulatory effect of herbicides on growth of Rhizobia

Inhibitory zones in mm and stimulation	Laboratory marking of strains												
	* Number and percent of the inhibitory (stimulatory) herbicides												
	* 1	2	3	4	5	6	7	8	9	10	11	12	13
Stimulation	—	—	—	—	2	—	2	—	—	—	1	—	—
	—	—	—	—	1.92	—	1.92	—	—	—	0.96	—	—
No	19	11	14	14	20	6	19	12	6	15	10	4	4
inhibition	18.24	10.56	13.44	13.44	19.2	5.76	18.04	11.52	5.76	14.40	9.60	3.84	3.84
Inhibition	11	4	5	6	17	1	12	2	0	3	9	4	0
0—9 mm	10.56	3.84	4.80	5.76	16.32	0.96	11.52	1.92	0	2.88	8.64	3.84	0
Inhibition	47	44	38	44	42	35	38	37	5	34	46	38	61
10—29 mm	45.12	42.24	36.48	42.24	40.32	33.60	36.48	35.52	4.80	32.64	44.16	36.48	58.56
Inhibition	21	45	40	31	15	60	25	42	17	46	25	47	39.
30—120 mm	20.16	43.20	38.40	29.76	14.4	57.60	24.0	40.32	16.32	44.16	24.0	45.12	37.41
Inhibition	6	0	7	9	8	2	8	11	76	6	1	11	0
140 mm	5.76	0	6.72	8.64	7.68	1.92	7.68	10.56	72.96	5.76	0.96	10.56	0

Inhibitory zones in mm and stimulation	Laboratory marking of strains												
	* Number and percent of the inhibitory (stimulatory) herbicides												
	* 14	15	16	17	18	19	20	21	22	23	24	25	26
Stimulation	—	—	—	—	3	—	3	—	3	—	—	—	—
	—	—	—	—	2.88	—	2.88	—	2.88	—	—	—	—
No	5	1	11	7	5	8	13	16	2	24	19	12	14
inhibition	4.80	0.96	10.56	6.72	4.80	7.68	12.48	15.36	1.92	23.04	18.24	10.82	13.4
Inhibition	14	7	1	3	1	2	14	15	1	11	16	5	12
8 – 9 mm	13.44	6.72	0.96	2.88	0.96	1.92	13.48	14.40	0.96	10.56	15.36	4.80	11.52
Inhibition	68	35	34	34	54	46	48	43	64	53	46	35	58
16 – 29 mm	65.28	33.60	32.64	32.64	51.84	44.16	46.08	41.28	61.44	50.88	44.16	33.6	56
Inhibition	17	49	46	49	41	38	20	16	34	10	17	43	14
30 – 120 mm	16.32	47.04	44.16	47.04	39.36	36.48	19.2	15.36	32.64	9.6	16.32	40.28	13.4
Inhibition	0	12	12	11	0	10	6	2	0	6	6	9	6
140 mm	0	11.52	11.52	10.56	0	9.60	5.76	1.92	0	5.76	5.76	8.64	5.76

Table 3

Non-inhibitory herbicides of the investigated 104 preparations

Herbicides	Number and percent of non-inhibited strains														
	1 (3.8)	2 (7.6)	3 (11.4)	4 (15.2)	5 (19.0)	6 (22.8)	7 (26.6)	8 (30.4)	9 (34.2)	10 (41.8)	11 (45.6)	12 (38.4)	13 (50.0)	17 (64.6)	18 (68.4)
A 2591				+											
Aniten D											+				
Aquathol G														+	
Avadex	+														
Balan	+														
Betanol					+										
Camparol 1803									+						
Casoron 133		+													
Cotoran								+							
Cycocel I.		+													
Cycocel II.		+													
Dikonirt		+													
Dikotex 40		+													
Dachtal													+		
Dymid	+														
Embutor E														+	
R. Eptapur	+														
Gesagard 50		+													
Gesapax 50		+													
Gesaprim 50				+											
Gesaprim 1802			+												
Gesaran		+													
Gesatop 50							+								
Hoe 2904	+														

Hortox													+		
Hungazin DT													+		
Hyvar								+							
Igran 50						+									
Igran 2105												+			
Kloben										+					
MH 30								+							
Planavin 75															+
Potablan							+								
Prevenol		+													
Pyramin			+												
Reglone		+													
Sequestren Na_2 Zn	+														
Sinbar			+												
Sys 67 I.	+														
Sys 67 II.		+													
Telvar		+													
Tenoran											+				
TOK E 25							+								
Tribunil			+												
Tropotox	+														
Vegadex			+												
Vegiben									+						
Venzar														+	
Vernam	+														
WL 19805						+									
Hungaria Viratol		+													
Herbicides, %	8.64	12.48	4.8	1.92	2.88	2.88	1.92	1.92	1.92	0.96	1.92	1.92	1.92	1.92	0.96

+: no inhibition.

Table 4

The strongest inhibitory herbicides of the investigated 104 preparations

Herbicides	Number and percent of inhibited strains										
	1 (3.8)	2 (7.6)	3 (11.4)	5 (19.0)	7 (26.6)	10 (38.2)	12 (45.6)	14 (53.8)	15 (57.0)	17 (64.6)	20 (76.4)
Amitrol TL		+									
Aretit								+			
Atracil C								+			
Avadex									+		
Balan										+	
Banvel D	+										
Banvel M	+										
Basamid											+
Betanol	+										
Bexone									+		
Bidisin			+								
BNP	+										
Camparol 1803	+										
Campione						+					
Casoron						+					
Chlorflurazole							+				
Cotoran					+						
Cycocel				+							
Cycocel (liquid)				+							
Dachtal		+									
MP 58	+										
Tonitox		+									
Herbicides %	5.94	2.88	0.96	1.92	0.96	1.92	0.96	1.92	1.92	0.96	0.96

+: very strong inhibition.

LIST OF HERBICIDES USED IN OUR INVESTIGATIONS

A) CONTACT HERBICIDES

I. *Nitro-compounds:*

1. BNP 20: 2,4-dinitro-6-s-butylphenol 20% Dinoseb
2. DNBP 20: 2,4-dinitro-6-s-butylphenol
3. Hoe 2904: 2,4-dinitro-6-s-butylphenol acetate
4. Aretit: 2,4-dinitro-6-s-butylphenol acetate
5. Amine 20%: DNBP solution
6. Raphatox: 2-methyl-4,6-dinitrophenol

II. *Chlorinated phenols:*

7. TOK E—25: 2,4-dichlorophenyl-4-nitrophenyl ether

III. *Bipyridylium derivatives:*

8. Gramoxone: 1-1'-dimethyl-4,4'-bipyridylium-dichloride
9. Reglone: 1,1'-methylene-2-bipyridylium dibromide 20%

B) SYSTEMIC HERBICIDES

Phenoxy-alkyl-carboxylic acids and their derivatives

IV. *2,4,5-trichloro-phenoxy acetic acid and its esthers:*

10. Embutor E:	butylesther of 2,4 DB
11. Trifenox 80:	2,4,5-trichlorophenoxyacetic acid
12. Sys 67/B:	4,(2,4-dichlorophenoxy)butyric acid
13. Legumex D:	4,(2,4-dichlorophenoxy)butyric acid

V. *2,4-dichlorophenoxy acetic acid and its salts and esthers:*

14. Lironox:	2,4-D amine-salt
15. Dikonirt:	2,4-dichlorophenoxyacetic acid

VI. *Chloro-crezoxy-acetic acid salts:*

16. M 52:	K-salt of MCPA
17. Dikotex 40	4-chloro-2-methylphenoxyacetic acid

VII. *Phenoxy propionic acid derivatives:*

18. Sys 67 I:	(±)-2-)4-chloro-2-methylphenoxy)propionic acid
19. Sys 67 II:	(±)-2-(4-chloro-2-methylphenoxy) propionic acid
20. MP 58:	K-salt of mecoprop

VIII. *Other phenoxy derivatives:*

21. Tropotox:	4-(2-methyl-4-chloro-phenoxy)-butyric acid
22. Dyserbo:	dichloro-phenoxyethanol
23. Bexone:	4-(4-chloro-2-methylphenoxy)-butyric acid
24. Legumex M:	4-(4-chloro-2-methylphenoxy)-butyric acid

Halogen derivatives of benzoic acid

IX. *Other substituted halogenic acids:*

25. Vegiben:	3-amino-2,5-dichlorobenzoic acid 10.8%

X. *Halogenized aliphatic carbonic acids:*

26. Bidisin:	2-chloro-3-(4-chloro-phenyl)-propionic acid methyl-ester

Aliphatic carbonamides

XI. *Alpha-chlor-acetamides:*

27. Legurame:	phenyl-carbamoyl-oxy-2-N-ethyl propion amide

XII. *Anilides:*

28. Potablan:	alfa,alfa-dimethyl valerianic acid-4-chloro-anilide
29. Ramrod:	2-chloro-N-isopropyl-N-phenylacetamide
30. Stam F 34/A:	N-(3,4-dichlorophenyl propionamide
31. Stam F 34:	N-(3,4-dichlorophenyl propionamide
32. Dymid:	N N-dimethyldiphenylacetamide
33. Hortox:	N'-(3-chloro-4-methylphenyl)-2 methylpentane-amide (46.50%)

Carbamates

XIII. *Phenyl-carbamates:*

34. Prevenol conc.:	isopropyl-N-(3-chlorophenyl)carbamate
35. Hungaria CIPC:	isopropyl-N-(3-chlorophenyl) carbamate
36. Betanal:	3 methoxy-carbonyl-amino-phenyl-N-(3'-methyl-phenyl) carbamate

XIV. *Thiocarbamates:*

37. Tillam 6 E: S-propyl-N-butyl-N-ethyl(thiocarbamate)
38. Vernam: S-propyl-N-N'-dipropyl-(thiocarbamate)
39. Avadex: S-2,3-dichloroalkyl-N-N'-diisopropyl(thiocarbamate)
40. Vegadex: diethyl-dithio-carbamate acid-chloroalkyl-ester 46.4%
41. Basamid: 3,4,5,6-tetrahydro-3,5-dimethyl-1,3,5-thiadiazine-2-thione
42. Campione: (EPTC) S-ethyl-N'N-dipropyl (thiocarbamate)
43. Ordram: S-ethyl-hexahydro-1-H-azepine-1-carbothioat

XV. *Urea derivatives:*

44. Telvar: N'-(4-chlorophenyl)-N-N'-dimethylurea
45. Cotoran: N-(3-trifluoromethyl phenyl)-N-N'-dimethylurea
46. Aresin: N'-(4-chlorophenyl)-N-methoxy-N-methylurea
47. Patoran: N'-(4-bromophenyl)-N-methoxy-N-methylurea
48. Linuron: N-(3,4-dichlorophenyl)-N'-methoxy-N-methylurea (50%)
49. Afalon: N-(3,4-dichlorophenyl)-N'-methoxy-N-methylurea
50. R. Eptapur: N'-(4-chlorophenyl)-N-isobutynyl-N-methylurea
51. Kloben: N-butyl-N'-(3,4-dichlorophenyl)-N-methylurea (60%)
52. Tenoran: N'-4-(4-chlorophenoxy)-phenyl-N-N'-dimethylurea
53. DCU I: N'N-bis-(2,2,2-trichloro-1-hydroxy-ethyl)urea
54. DCU II: N'N-bis-(2,2,2-trichloro-1-hydroxy-ethyl)urea
55. Tribunil: N'N-dimethyl-N'-(2-benzthiazolyl)urea

XVI. *Benzo-nitrile and thioamide derivatives:*

56. Casoron 133: 2,6-dichlorobenzonitrile
57. Treflan: 2,6-dinitro-N'N-dipropyl-4-trifluoro-methyl aniline
58. Prefix: 2,6-dichlorothiobenzamide
59. NPH/1264: K-salt of 3,5-dibromo-4-hydroxybenzonitrile
60. NPH/1254: Na-salt of ioxynil

XVII. *Phthalic acid esters:*

61. Dachtal: dimethyl 2,3,5,6-tetrachloroterephthalate (75% t-t. acid)

Different cyclic carbonic acid derivatives

XVIII. *Hexa-hydro-phthalate:*

62. Hydrathol: 7-oxabicyclo(2,2,1)-heptane-2,3-dicarboxylic acid
63. Aquathol G: 7-oxabicyclo(2,2,1)-heptane-2,3-dicarboxylic acid

XIX. *Triazine derivatives:*

64. WL 19805: 2-(4-chloro-6 ethyl-amino-S-triazine-2-yl amino)2 methyl-propionitrile

XX. *Alkyl-amino-chloro-triazines:*

65. Hungazin DT: 2-chloro-4,6-bisethylamino 1,3,5-triazine
66. Gesatop 50: 2-chloro-4,6-bisethylamino 1,3,5-triazine
67. Gesaprim 50: 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine
68. A 2591: 4-aethylamino-2s-butylamino-6-methoxy-s-triazine

XXI. *Mercapto-triazines:*

69. Gesagard 50: 2,4-bisisopropylamino-6-methylthio-1,3,5-triazine
70. Semeron: 2-isopropylamino-4-methylamino-6-methylthio-1,3,5--triazine
71. Gesapax 50: 2-ethylamino-4-isopropylamino-6-methylthio-1,3,5--triazine
72. Igran 50: 2-ethylamino-4-methylthio-6-t-butylamino-1,3,5--triazine
73. Igran 2105: 2-ethylamino-4-methylthio-6-t-butylamino-1,3,5--triazine
74. Merkazin: 2,4-bisisopropylamino-6-methylthio-1,3,5-triazine

Other herbicidal active ingredients:

XXII. *Heterocyclic compounds:*

75. Amitrol TL:	3-amino-1,2,4-triazolonium-rodamide
76. Amino Triazole:	3-amino-1,2,4-triazole
77. Sinbar:	5-chloro-6-methyl-3-t-butyluracil (80%)
78. Venzar:	3-cyclohexyl-5,6-trimethyleneuracil
79. Hyvar:	5-bromo-6-methyl-3-s-butylacril (80%)
80. Pyramin:	5-amino-4-chloro-2-phenylpyridazin-3(2H)-one

XXIII. *Balan: N-butyl-N-ethyl-2,6-dinitro-4-trifluormethylalanilin*

82. Planavin 75:	2,6-dinitro-4-methylsulphonyl-N-N'-dipropylanilin (75%)

C) OTHER HERBICIDES

XXIV. *Regulators not auxin type:*

83. Cycocel I:	(chloro-cholin-chloride)
84. Cycocel II:	(chloro-cholin-chloride)
85. MH 30:	1,2-dihydropyridazine-3,6-dione (malein-hydrazid)

XXV. *Substitutives for lack of trace elements:*

86. Sequestren 138 Fe:	EDDHA-NaFe (6% Fe)
87. Sequestren Na_2Zn:	EDTA-Na_2Zn (14.2% Zn)

XXVI. *Combinations:*

88. Aniten D:	2,4-D + Flurenol (as dimethylamino salts)
89. Aniten M:	MCPA + Flurenol (as dimethylamino salts)
90. Atracil C:	ioxynil + mecoprop as the potassium salts
91. Banvel D:	2-methoxy 3,6-dichloro-benzoic-acid + 2-4 D
92. Banvel M:	2-methoxy-3,6-dichloro-benzoic-acid + MCPA
93. Camparol 1803:	Promethryne + Simazine
94. Chlorflurazole: XB:	Chlorflurazole + MCPA
95. Embutox plus:	2,4-DB + MCPA
96. Gesaran:	2-isopropylamino-4(3-methoxypropilamino)-6-methyl-thio-1,3,5-triazine + Simazine
97. Gesaprim 1802:	Ametrine + Atrazine
98. Hoe 2849:	Dinoseb-acetate + monolinuron
99. Hungaria Viratol:	Aktinit PK 20% + Merkazin 8% + Sazol 15% Dikonirt 15% + inert material
100. Liro-Betarex:	IPC + Diuron
101. Murbetol:	Endothal + propham
102. Saminol:	Simazine + amitrole
103. Tonitox:	Aktinit PK (20%) + chlorophenoxyacetic acid (35%) + Krezonit E (3%) + inert material
104 Tropotox plus:	MCPB + MCPA Sodium salts (30%)

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SENSITIVITY OF ROOT-NODULE BACTERIA TO DIFFERENT SEED PROTECTANTS

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The use of compounds, either in powdered or liquid forms, to combat plant diseases is a wel-established practice. Since the report of Hiltner and Strömer (1903 a and b) there have appeared a number of papers, emphasizing the value of these various substances for desinfecting seeds or soils, (Fred *et al.* 1932).

In relation to legume seed inoculation, the use of these seed desinfectants provides a problem. At first as pointed out by Robinson (1910) it was impracticable to treat the seed with a strong poisonous agent and immediately afterwards to add a pure culture of bacteria or vice versa. Obviously, such a treatment would injure or destroy the bacteria. Therefore cup plate technique was used in this phase of study to show the sensitivity of the different *Rhizobium* strains towards the seed protectants used in UAR.

MATERIAL AND METHODS

Forty-one strains of rhizobia representing 7 *Rhizobium* species from the Department of Soil and Water Science, University of Alexandria collection were used in this study. All strains were maintained on slopes of yeast mannitol agar (Allen 1959).

Ethyl alcoholic solution of the seed protectants:

1. Antracol: (zinc-1,2-propylene-bis-dithiocarbamate)
2. Karathane: (2,4-dinitro-6-(2-octyl)phenyl crotonate)
3. Captan: (N-trichloro-methylmercapto-4-cyclohexene-1,2-dicarboximide)
4. Ceresan Wet: (Ethyl-mercury-2,3-dihydroxy-propyl-mercaptide)
5. Spergon: (2,3,5,6-tetra-chloro-1,4-benzoquinone)
6. Thiram: [tetramethyl-thiuram-disulphide-bis-(dimethyl thiocarbamyl)] disulphide
7. Dithan M 45: (A Co-ordination product of zinc ion and manganese ethylene-bis-dithiocarbamate)
8. Ceredon-T: (quinone-oxime-benzoyl-hydrazone)
9. Ceresan: (ethyl-mercury-2,3-dihydroxy-propyl-mercaptide)
10. Rhizoctol: (methyl-arsenic-sulphide)

were used to test their toxic effect. The cup plate method was used to test the toxic effect. Control plates, using only alcohol instead of the alcoholic solution of the seed protectants, for each rhizobial strain, were used.

RESULTS AND DISCUSSION

Preliminary studies on the effect of seed protectants on the growth of root-nodule bacteria showed that:

1. Ethyl alcohol solutions were more suitable for the used assay cup method than solutions of water or other solvents.
2. Measurable inhibition zones were obtained by using 50,000 p.p.m. of Antracol and Karathane and 10,000 p.p.m. of the other tested seed protectants.

The data presented in Tables 1, 2 and 3 and Figs 1, 2 and 3 show the size of inhibition zones obtained upon assaying the ten seed protectants, against the 41 strains of rhizobia which represented 7 cross-inoculation groups by the cup-plate method. In general, Karathane was the least toxic seed protectant among the tested groups with respect to the used technique. Although Antracol and Karathane were used in higher concentrations, they were less effective than the others. The tested seed protectants could be arranged according to the effect of the used concentrations and the diameter of inhibition zones as follow:

Ceresan Wet, Thiram and Ceredon-T > Thizoctol and Ceresan > Captan and Dithan M 45 > Spergon > Antracol > Karathane.

Table 1

Effect of seed protectants on growth of Rhizobium meliloti and Rhizobium trifolii using the assay cup method

Species and strains	Diameter of inhibition zones in mm with seed protectants*									
	Antracol	Karathane	Captan	Ceresan wet	Spergon	Thiram	Dithan M 45	Ceredon—T	Ceresan	Rhizoctol
Rh. meliloti										
107	27	35	26	46	12	55	12	33	18	—
112	28	59	29	55	21	53	25	54	45	31
Rh. trifolii										
202	15	27	15	11	—	33	15	33	15	19
203	15	—	—	24	13	—	—	—	17	34
215	29	—	20	36	—	25	10	25	17	25
216	14	—	18	38	—	25	25	29	34	22
226	12	—	12	43	—	28	12	26	22	20
233	12	—	12	38	—	22	11	18	23	22
236	—	—	16	25	—	22	—	18	16	20
239	28	—	20	36	—	17	—	20	22	—
240	25	—	12	29	—	15	—	19	90	90

* Concentration of Antracol and Karathane was 50 000 p.p.m, others 1000 p.p.m.
— No inhibition.

Table 2

Effect of seed protectants on growth of Rhizobium phaseoli using the assay cup method

Species and strains	Diameter of inhibition zones in mm with seed protectants*									
	Antracol	Karathane	Captan	Ceresan wet	Spergon	Thiram	Dithan M 45	Ceredon - T	Ceresan	Rhizoctol
Rh. phaseoli										
415	—	32	22	28	12	49	34	61	22	—
416	36	—	25	36	15	28	27	30	33	20
417	24	25	23	36	20	46	28	54	34	22
456	—	—	14	28	—	36	11	27	12	16
462	19	—	—	40	—	30	16	25	29	11
464	12	—	—	36	—	14	9	18	21	36
467	—	—	12	21	—	24	—	24	—	29
468	20	—	21	22	18	27	18	25	90	32
469	23	—	15	33	12	16	18	21	30	37
473	33	16	14	28	9	45	14	14	30	30
476	14	24	25	40	11	46	17	41	30	33
477	25	—	15	66	—	47	30	56	60	19
483	16	—	11	45	—	38	15	37	40	16
485	38	—	17	43	11	43	32	36	50	23
487	30	—	30	55	11	50	30	45	36	37
493	15	—	—	14	—	—	—	—	—	14

* Concentration of Antracol and Karathane was 50,000 p.p.m, others 1000 p.p.m.
— No inhibition.

The results are of particular interest, in that a marked antibiotic activity, was shown by seed protectants against certain rhizobial strains. Hofer *et al.* (1956) and Vintikova *et al.* (1963) noticed that rhizobia differed somewhat in their sensitivity to herbicides.

This investigation indicated also that the severity of seed protectants to rhizobia might be lessened by proper selection of rhizobia strains and seed protectant. It is conceivable that resistance to seed protectants may partially determine success of *Rhizobium* strains in a mixture during inoculation.

The sensitivity of the tested bacteria seemed to be a property of the strain and to some extent of the species.

SUMMARY

The seed protectants used in this phase of study were: — Antracol, Karathane, Captan, Ceresan-Wet, Spergon, Thiram, Dithan M 45, Ceredon-T, Ceresan and Rhizoctal.

Table 3

Effect of seed protectants on growth of Rhizobium leguminosarum, lotus bacteria, Rhizobium lupini and Rhizobium japonicum using the assay cup method

Species and strains	Diameter of inhibition zones in mm with seed protectant*									
	Antracol	Karathane	Captan	Ceresan wet	Spergon	Thiram	Dithan M 45	Ceredon–T	Ceresan	Rhizoctol
Rh. leguminosarum										
301	19	—	17	25	—	25	—	31	17	—
307	14	30	11	28	11	45	14	45	15	31
351	14	—	11	26	—	26	—	29	19	25
352	22	—	13	34	—	30	11	33	30	20
Lotus bacteria										
701	20	25	15	48	—	20	18	39	11	—
704	—	—	15	25	—	26	—	26	—	25
709	—	22	12	22	—	17	11	17	16	—
712	12	—	13	32	—	17	—	18	13	22
713	29	25	18	50	15	42	24	42	54	27
Rh. lupini										
804	26	25	14	52	15	28	19	26	29	17
558	12	—	—	27	—	37	—	35	12	—
Rh. japonicum										
505	11	—	—	15	—	—	—	10	—	—
507	12	30	—	—	—	30	—	10	—	22

* Concentration of Antracol and Karathane was 50,000 p.p.m., others 1000 p.p.m.
— No inhibition.

An ethyl alcohol solution was prepared from each seed protectant. The size of inhibition zones upon assaying the ten seed protectants against 41 strains of rhizobia representing 7 *Rhizobium* species by the cup method were obtained. Measurable inhibition zones were obtained by using 50.000 p.p.m of Antracol and Karathane and 1000 p.p.m of the other tested seed protectants.

In general, Karathane was the least toxic seed protectant among this group with respect to the used technique. Antracol and Karathane although used in higher concentrations were less effective than the others. The used ten seed protectants inhibited the growth of root nodule bacteria as tested by the plate cup technique. The seed protectants, could be arranged according to the effect of the used concentrations and the diameter of inhibition zones for rhizobia strains as follows:

Ceresan Wet, Thiram and Ceredon-T > Rhizoctol and Ceresan > Captan and Dithan M 45 > Spergon > Antracol > Karathane.

The sensitivity of the bacteria seemed to be a property of strain and to some extent of the species.

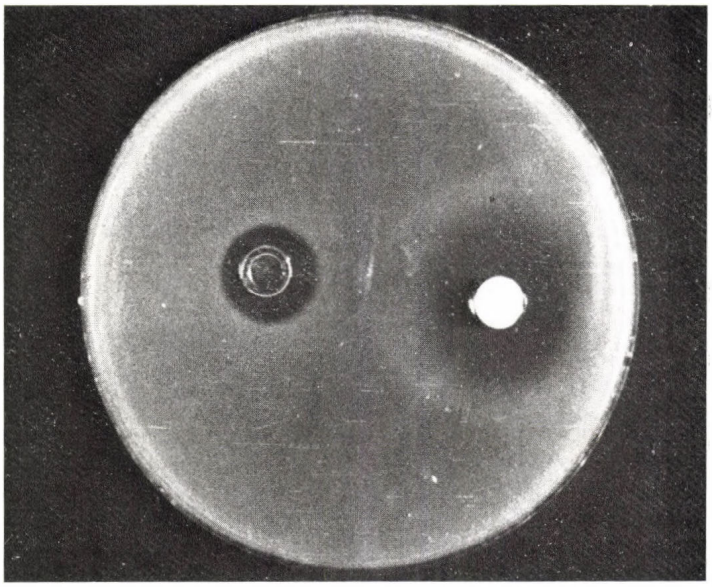
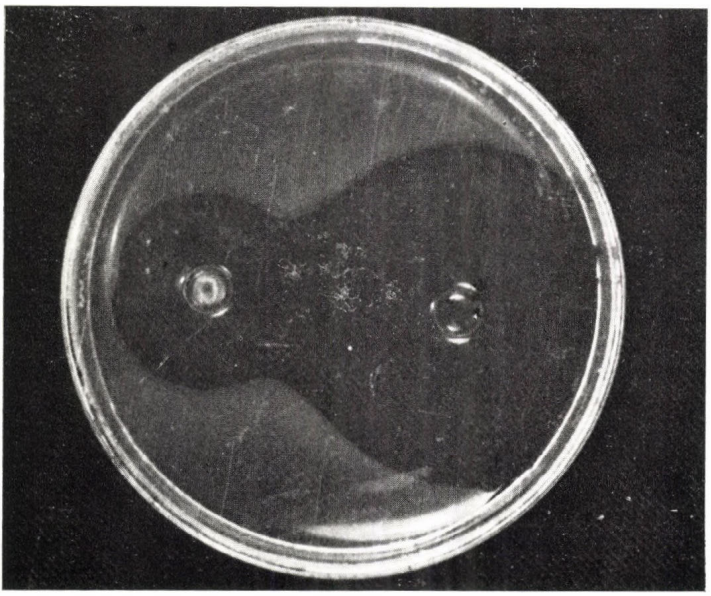


Fig. 1. Inhibiton of *Rhizobium trifolii* (strain 215) by seed protectants. *A)* Inhibition by 500,000 p.p.m. Antracol. *B)* Inhibition by 1000 p.p.m. Captan

Fig. 2. Inhibition of *Rhizobium phaseoli* (strain 417) by seed protectants. *A)* Inhibition by 1000 p.p.m. Dithan M 45. *B)* Inhibition by 1000 p.p.m. don-T-Cr



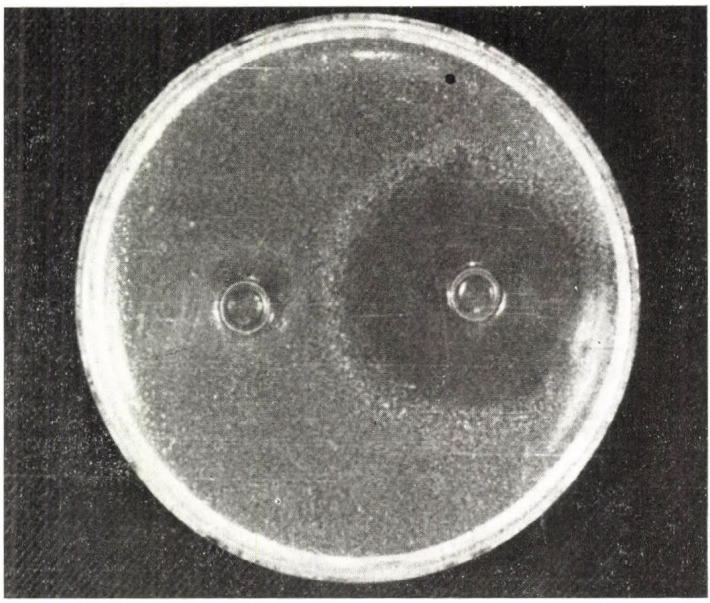


Fig. 3. Inhibition of *Rhizobium phaseoli* (strain 477) by seed protectants. *A)* Inhibition by 1000 p.p.m. Ceresan. *B)* Inhibition by 1000 p.p.m. Rhizoctal

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EFFECT OF PESTICIDES ON *RHIZOBIUM* INOCULATION, NODULATION AND SYMBIOTIC N-FIXATION OF SOME LEGUMINOUS PLANTS

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Pesticides are extensively used nowadays to combat harmful effects, pathogenic fungi and weeds. Since most of the organic pesticides are enzyme inhibitors, they are supposed to affect the soil and rhizospheric microflora including indigenous and inoculated rhizobia, when they reach the soil in considerable amounts. Some pesticides, however, were found to stimulate, rather than inhibit the soil microflora.

The stimulating effect of herbicides and insecticides on nodulation was recorded by some investigators such as Anderson and Baker (1950), Abou El-Fadl and Fahmy (1958). They found that 2,4-D and DDT had a stimulatory effect on the formation of nodules of some leguminous plants. Taha *et al.*(1966) showed also a stimulating effect of Dipterex on the formation of many effective nodules of broad bean and Egyptian clover. On the other hand, other investigators showed that herbicides and insecticides might inhibit rhizobia or nodulation process, among them were Payne and Fults (1947), Nilsson (1957) and Braithwaite *et al.* (1958). Taha *et al.* (1966) studied another herbicide (CIPC) and they found that it completely inhibited nodulation in both broad bean and clover.

The effect of fungicides on the nodulation process was studied by some investigators such as Duggar (1935), Appleman (1941), Kernkamp (1948). They showed that application of fungicides in field practice caused no deleterious effect on *Rhizobium* present in the soil and nodulation of legume plants. Others found that mercuric fungicides inhibited nodulation of leguminous plants or the root nodule bacteria, among them were Kadow *et al.* (1937), Appleman (1941), Ruhloff and Burton (1951), Peterson and Buchholtz (1955), Hofer (1958), Jakubisiak and Gołebiowska (1964) and Gołebiowska (1966). They found that mercuric preparations were highly toxic to fungi and nodule bacteria of pea, clover and cowpea.

These views demonstrated that, there were some contradictions in the data obtained by different investigators depending on the chemical formula of the pesticide, its concentration, its mode of action and the sensitivity of rhizobia to these materials.

As little data were available on the effectiveness of inoculation following pesticide treatments under Egyptian conditions, it seemed desirable to investigate the influence of applying insecticides and fungicides in field practice on nodulation and rate of N-fixation in some leguminous plants.

MATERIAL AND METHODS

Pot experiments were carried out with sterilized and non-sterilized soil in the greenhouse. The soil was fertile clay loamy soil, from the College Farm at Shoubra El-Khaima. Three soil treatments were used, namely inoculated sterilized soil, inoculated non-sterilized soil, and uninoculated non-sterilized soil. Two leguminous plants broad bean *(Vicia faba)* and lentil *(Lens esculenta)* were used in this investigation. They had been cultivated in November 1965. Each pot was sown with 7 seeds of broad bean or 12 seeds of lentils. After 15 days from sowing, the plants were thinned to leave 4 and 8 plants per pot in case of broad bean and lentil respectively. Plants were irrigated every 2 days with sterile tap water.

The following insecticides and fungicides were used:

I. Insecticides:

a) Dipterex: 0,0-Dimethyl-1-hydroxy-2,2,2-trichloro-ethyl-phosphanate (80% active ingredient) at rate of 1.5 kg/ feddan.

b) Endrin: Hexa-chloro-phenoxy-octahydron (19.5% active ingredient) at a rate of 2 kg/feddan.

All insecticides were sprayed by atomizer after cultivation of inoculated seeds.

II. Fungicide:

Ceresan: Ethyl-mercury-phosphate; at a rate of 0.5% per seed weight. The fungicide was applied as seed dressing and the inoculation with an appropriate effective strain of rhizobia was carried out using atomizer to the soil after the treated seeds were sown.

In case of insecticides, the pots were divided into 3 sets. The first set received one spray of the insecticide 10 days after emergence, the second set received 2 sprays after 10 and 20 days after emergence while the third one received 3 sprays 10, 20 and 30 days after emergence.

After 45 days, the nodules on every plant root system were counted, and the efficient nodules were sorted according to their size and location on the root. Dry weight of the whole plants was also determined. Total nitrogen was measured in dried plants using the modified micro-Kjeldahl method as a criterion of nitrogen fixation.

RESULTS

Broad bean plants: Table 1 shows the action of pesticides on the nodulation, dry weight and nitrogen fixation in broad bean plants. The data showed that application of Dipterex resulted in a sligth increase in the number of both big and total nodules. It was also generally observed that the highest number of big nodules was obtained on plants in sterilized soil when they received two sprays. In sterilized inoculated soil, for example, the densities of big nodules on the roots were 71, 75, 96 and 79 nodules/plant for treatments which had received (control), 1, 2 and 3 sprays of dipterex respectively. The respective values of total nodules were 156, 168, 176 and 162 nodules/plant. Non sterilized soil either inoculated or uninoculated gave the same general trend. These data indicated that Dipterex had a slight stimulatory effect on nodulation capacity of broad bean plants.

Table 1

Effect of pesticides on nodulaltion, dry matter and nitrogen content of broad bean plants (Vicia faba)

Treated soil	Pesticides	Spraying	Count of nodules/plant			Dry wt.* g/pot	T. N.** mg/pot
			small	big	total		
	Control	None	85	71	156	5.63	196.49
Inoculated sterilized soil	Dipterex	1 spray	93	75	168	5.74	216.91
	Dipterex	2 sprays	80	96	176	5.87	259.07
	Dipterex	3 sprays	83	79	162	5.87	244.21
	Endrin	1 spray	83	70	153	5.76	225.63
	Endrin	2 sprays	90	62	152	5.32	189.08
	Endrin	3 sprays	85	47	132	5.40	153.49
	Ceresan	Seed dressing	65	39	104	3.98	146.91
	Control	None	83	102	185	6.41	224.19
Inoculated non-sterilized soil	Dipterex	1 spray	81	116	197	6.53	235.64
	Dipterex	2 sprays	71	128	199	6.96	317.38
	Dipterex	3 sprays	101	115	216	6.51	267.69
	Endrin	1 spray	101	118	219	6.50	225.93
	Endrin	2 sprays	86	121	207	6.96	299.56
	Endrin	3 sprays	73	139	212	8.19	352.71
	Ceresan	Seed dressing	56	89	145	5.65	218.94
	Control	None	32	63	95	5.44	186.70
Uninoculated non-sterilized soil	Dipterex	1 spray	41	69	110	6.08	209.74
	Dipterex	2 sprays	69	84	153	6.53	285.52
	Dipterex	3 sprays	42	81	123	6.93	221.73
	Endrin	1 spray	49	66	115	6.37	233.07
	Endrin	2 sprays	54	88	142	6.89	328.72
	Endrin	3 sprays	34	64	98	6.27	196.82
	Ceresan	Seed dressing	15	46	61	4.09	172.41

* = Dry weight.
** = Total nitrogen.

Application of Dipterex also showed a slight increase in the plant growth as indicated by the dry matter of the plants. This was illustrated in the three soil treatments, namely sterile inoculated, non-sterile inoculated and non-sterile non-inoculated soils. The total nitrogen content increased by the application of Dipterex. The increase was more pronounced in plants which received 2 sprays. Total nitrogen was found to represent 216.91, 259.07 and 244.21 mg/pot for plants received 1, 2 and 3 sprays respectively under sterile inoculated conditions. This coincides with the data of big nodule counts and dry weight.

Unlike Dipterex, Endrin treatment was found to show a decrease in the density of large and total nodules on the root system of treated plants in sterilized inoculated soils. The inhibitory effect increased with the number of sprays. In non-sterilized inoculated and non-inoculated soils, however,

the figures of big nodules were higher than the control. In case of dry weight effect, the same general trend was observed in nearly all treatments. With regard to nitrogen fixation, it was generally found that there was a considerable decrease in the nitrogen content in case of additional 2 or 3 sprays of Endrin to sterilized soil. In non-sterilized inoculated soil, on the other hand, the nitrogen content was found to be the highest in the treatments which received 2 or 3 sprays. This indicated that the presence of the normal microflora is very important for the degradation of Endrin and thus decreases its toxicity.

Application of Ceresan as seed dressing resulted in considerable decrease in the number of big and total nodules. The effect was more pronounced under sterile soil conditions. The density of big nodules was 39, 89 and 46 nodules/plant for sterile inoculated, non-sterile inoculated and non-

Table 2

Effect of pesticides on nodulation, dry matter and nitrogen content of lentil plants (Lens esculenta)

Treated soil	Pesticides	Spraying	Count of nodules/plant			Dry wt.* g/pot	T. N.** mg/pot
			small	big	total		
	Control	None	12	13	25	0.819	24.59
Inoculated sterilized soil	Dipterex	1 spray	22	19	41	1.028	33.34
	Dipterex	2 sprays	22	13	35	0.945	25.62
	Dipterex	3 sprays	21	12	33	0.710	22.78
	Endrin	1 spray	16	14	30	0.837	25.76
	Endrin	2 sprays	17	14	31	1.058	26.16
	Endrin	3 sprays	20	16	36	1.117	28.83
	Ceresan	Seed dressing	17	3	20	0.520	9.51
	Control	None	32	8	40	0.829	26.88
Inoculated non-sterilized soil	Dipterex	1 spray	34	12	46	0.837	30.26
	Dipterex	2 sprays	40	15	55	1.025	36.49
	Dipterex	3 sprays	40	19	59	1.431	42.50
	Endrin	1 spray	32	13	45	0.915	27.39
	Endrin	2 sprays	35	14	49	1.011	38.48
	Endrin	3 sprays	37	21	58	1.636	44.73
	Ceresan	Seed dressing	10	8	18	0.665	19.42
	Control	None	20	8	28	0.799	19.14
Uninoculated non-sterilized soil	Dipterex	1 spray	25	11	36	0.822	21.40
	Dipterex	2 sprays	24	12	36	1.174	23.79
	Dipterex	3 sprays	20	14	34	1.339	28.14
	Endrin	1 spray	16	10	26	0.849	23.28
	Endrin	2 sprays	26	11	37	0.917	24.86
	Endrin	3 sprays	29	13	42	1.021	27.74
	Ceresan	Seed dressing	18	9	27	0.747	15.61

* = Dry weight.
** = Total nitrogen.

sterile non-inoculated soil respectively. The respective values for untreated plants were 71, 102 and 63 nodules/plant. Ceresan caused also a considerable reduction in dry weight per pot. This effect might be a reflection of the effect of Ceresan on nodulation. Total nitrogen content was also lower than the control in all treated soils.

Lentil plants: data in Table 2 shows that Dipterex generally resulted in considerable increase of the number of big nodules in the three soil treatments. In sterile soil, application of 2 or 3 sprays resulted in the formation of nearly the same number of big nodules, being in the same order as the control. Total nodules per plant were similarly stimulated. The dry weight and total nitrogen content of the plants were also enhanced except in the case of plants which received 3 sprays in sterile soil.

When Endrin was used, it gave almost similar results to Dipterex. The number of big nodules was found to be 16, 21 and 13 for plants receiving 3 sprays in sterile inoculated, non-sterile inoculated and non-sterile uninoculated soils respectively. This effect was reflected on the dry weight and total nitrogen of the plants, similar to the general trend obtained in case of broad bean.

Ceresan treatment resulted in an appreciable reduction of the density of big and total nodules, dry weight and total nitrogen content. The effect was more pronounced in sterile soil.

DISCUSSION

Application of Dipterex caused a stimulatory effect on the formation of both big nodules, dry weight and total nitrogen content of broad bean and lentil plants i.e. increased the nitrogen fixation capacity of these plants. This stimulatory effect was more pronounced in case of non sterilized soils. Such result supports the previous work of Taha *et al.* (1966), who showed stimulatory effect of Dipterex application on the formation of higher numbers of efficient nodules in broad bean and Egyptian clover plants. The stimulatory effect observed in this work in non-sterile soil could be due to the degradation of Dipterex, the organophosphorus insecticide by soil microflora with the liberation of inorganic phosphorus. Most probably the latter might enhance plant growth. Verona and Picci (1952) also came to the same conclusion. It also confirmed the results of Illey (1963) and Lichenstein and Schulz (1964).

The stimulatory effect of Endrin on nodulation and nitrogen fixation of broad bean and lentil plants, obtained by these data, was in accordance with that found by Taha *et al.* (1966) on studying Egyptian clover. Contrary to the previous results those obtained by Braithwaite *et al.* (1958) who found that nodulation of clover was slightly reduced on the application of Endrin, Dieldrin or Chlordane.

In case of broad bean plants, the increase in the number of Endrin sprays caused a stimulatory effect on the count of big nodules up to the second spray 20 days after cultivation especially in non-sterile soil. In the treatment of 3 sprays, the stimulatory effect was relatively lower than that which received only 2 sprays. This might be due to the accumulation of some insecticides in the soil which have not yet been decomposed. In case of

lentil plants, the increase in the number of nodules continued till the third spray. This may be deduced to the difference in susceptibility to toxicants between different strains of rhizobia. This confirmed the results obtained by Hofer (1958) and Brakel (1963).

When Ceresan was used as seed dressing in the current work, it caused a considerable inhibition to nodulation on the plants especially in sterilized soil. It should be kept in mind that when fungicides are used as seed dressing, their concentrations in the root region will eventually be high and this deleteriously affects the normal microflora including rhizobia. This finding is in accordance with Appleman (1941), Hofer (1958), Jakubisiak and Gołebiowska (1964), Gołebiowska (1966). On the other hand, some investigators reported that application of fungicides in the field caused no deleterious effect on rhizobia in soil and nodulation of some leguminous plants, such as Dugger (1935), and Kernkamp (1948).

In a similar study Hamed (1968) showed an increase in number of nodules and plant growth in Orthocide treatments (fungicide) in lentil and broad bean plants. The stimulatory effect obtained in his data, but not recorded in this investigation, in case of using the fungicide "Ceresan" might be due to the liberation of NH_4^+ ions from the orthocide enhancing the growth of leguminous plants which are known to require nitrogenous compounds in early stages of growth for good nodulation (Taha *et al.* 1967). It should be borne in mind that Ceresan is a chemical compound (ethyl mercury phosphate), containing no nitrogen. Therefore, its decomposition does not liberate any nitrogen as Orthocide.

It can be concluded from the above discussion that when insecticides are used at normal field rates, they have no deleterious effect on nodulation and symbiotic N-fixation process. Fungicides although they may retard the process, due to their ability to combat fungi, they should be applied to the legumes with care. It would be advisable to inoculate the soil with rhizobia after a time sufficient to decompose the chemical.

SUMMARY

The effect of some pesticides on nodulation and N-fixation in leguminous plants was studied in pot experiments. These experiments were carried out under sterilized and non-sterilized soils.

The study revealed the following conclusions:

1. Application of Dipterex or Endrin in normal field rates was found to stimulate a high number of effective nodules and high rate of N-fixation especially under non-sterile soil conditions.
2. Fungicides represented by Ceresan caused a considerable decrease in the numbers of total and big nodules in both lentil and broad bean plants. The deleterious effect was reflected on the dry weight and nitrogen content of the plants. The effect was also more pronounced under sterile soil conditions.
3. It could be concluded that symbiotic N-fixation process was not affected by normal field application rates of insecticides, while fungicides retarded the process. Therefore, rhizobial inoculation in the latter case should be added to the soil after a time sufficient to decompose the chemical.

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THE EFFECT OF SOME SEED TREATMENTS USING MICROELEMENTS AND FUNGICIDES ON VETCH PLANTS GROWN FROM SEEDS INOCULATED WITH RHIZOBIA

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As it is well known the metabolism of higher plants and microorganisms can be influenced intensively by microelements: Mo in the atmospheric N-fixation and its incorporation into the different compounds, Mn in the photosynthesis and N-metabolism and B is essential in reproduction etc. (Skolnik 1960; Bersin 1963; Peyve 1964; Vlasyuk 1965; Vlasyuk 1966; Coppenet 1968; Peyve 1969). In the course of our investigations conducted earlier, Thiuram had a favourable effect on the yield of vetch and Ceresan decreased it in the light room, glasshouse and field. Kecskés and Vincent 1969b; Kecskés and Vincent (in manuscript).

Considering these, we aimed to study the effect of the microelements and fungicides as well as their combinations on the development and nutrient content of vetch *(Vicia sativa L.)* test plant as well as its symbiosis with rhizobia and on the growth of rhizobia.

MATERIALS AND METHODS

Pot experiments were carried out with molibden (Mo) boron (B) and manganase (Mn) microelements as well as Thiuram and Ceresan fungicides (Kecskés and Vincent 1969a) in slightly acid forest soil of Nagykálló and carbonaceous sand (rust coloured forest soil) of Őrbottyán. 96 pots were used in both experiments, 6 plants in 2 kg soil/pot. The microelements were applied with wet seed dressing, while fungicides in the form of dust. The microelements and fungicides were used separately and combined. There were 12 treatments, in quadruplications, in both cases, in 2 series each. Treatments of No. 2 were identical with No. 1 but they were inoculated with Rhizonit (vetch rhizobia- containing inocula manufactured in Hungary).

The wet and dry weight as well as macro- and micro-nutrient composition (total N, P, K and B and Mn content) of the harvested plants were measured. Their protein content was determined too. After carefully washing the roots, the position and number of nodules were observed on the basis of root nodulation forms (Kecskés and Vincent 1969c).

In the course of laboratory investigations, the inhibitory effect of investigated fungicides, on four strains of *Rhizobium leguminosarum* Frank et emend., Baldwin and Fred was established with filter paper disc method (details in paper of Kecskés and Vincent 1969a).

Strains came from four different countries: TA 101 (Australia UDALS, University of Sydney) B 8 (Bulgaria "N. Pushkarov" Institute of Soil Science, Sofia) D 1 (Czechoslovakia, Czechoslovak Collection of Microorganisms CRIPP Prague—Ruzyně (BO) Hungary, Phylaxia State Serum Institute Budapest).

The effect of treated vetch seeds (with microelements and fungicides separately and combined) on SU 391 strain of *Rhizobium leguminosarum* was also examined.

RESULTS AND DISCUSSION

The treatments used in the experiments increased the dry matter weight of vetch with 2—31% in the case of inoculated series (Table 1). This increasing effect was significant in a few cases. In the majority of treatments

Table 1

Dry top weight of 10 vetch plants in g

Treatments	In slightly acid sand		In carbonacecus sand	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Control	3.22	3.83	0.84	1.12
B	3.45	3.28	1.00 ×	1.02
Mo	3.61	3.58	0.96	1.15
Mn	3.75	3.49	1.11 ×	1.17
C	3.07	3.14	0.99	1.13
T	3.96	3.31	1.09 ×	1.06
B + C	3.23	4.31	0.98	1.01
Mo + C	3.49	3.59	1.08 ×	1.11
Mn + C	3.30	4.06	1.10 ×	1.15
B + T	4.08	3.77	0.90	1.13
Mo + T	3.83	2.78	0.98	1.16
Mn + T	2.91	3.30	0.84	1.32 ×
			$SD_{5\%} = 0.16$	$SD_{5\%} = 0.15$

C = Ceresen
T = Thiuram

of inoculated series the dry matter production decreases compared with the control. It varied between 73—118% expressed in the percent of control.

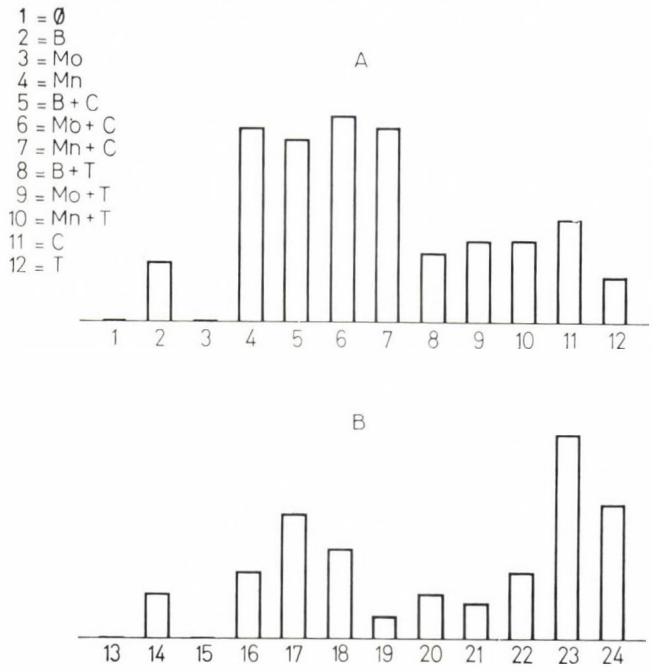
From the data of the nutrient material analysis (Table 2) — considering the whole experiment — no unequivocal conclusion could be drawn though in some cases significant differences could be recorded on the effect of certain treatments. Microelements applied separately in the experiments carried out in acidic forest soil did not change the total N and K content but decreased significantly that of the P. On the effect of fungicides the N and P content decreased, it did not change the K. The combined application of microelements increased the total N content and in some cases de-

Table 2

Vetch I investigation results

Treatment	N	P	K
	mg/100 g d. w.		
Control	3134	299	2740
B	3189	266 —	2590
Mo	3032	269 —	2530
Mn	3054	218 —	2760
C	2975 —	273 —	2660
T	2839 —	222 —	2780
B + C	4077 +	237 —	2070 —
Mo + C	3360 +	331 +	2560
Mn + C	3040	292	2390 —
B + T	3347 +	258 —	2040 —
Mo + T	3329 +	300	2320
Mn + T	3163	312	2430
	$SD_{5\%} = 124$	$SD_{5\%} = 13$	$SD_{5\%} = 325$

Fig. 1. Protein content of vetch in control per cent. Microelements: B, Mo, Mn. Fungicides: C = Ceresan, T = Thiuram. *A)* Uninoculated series. *B)* Inoculated series



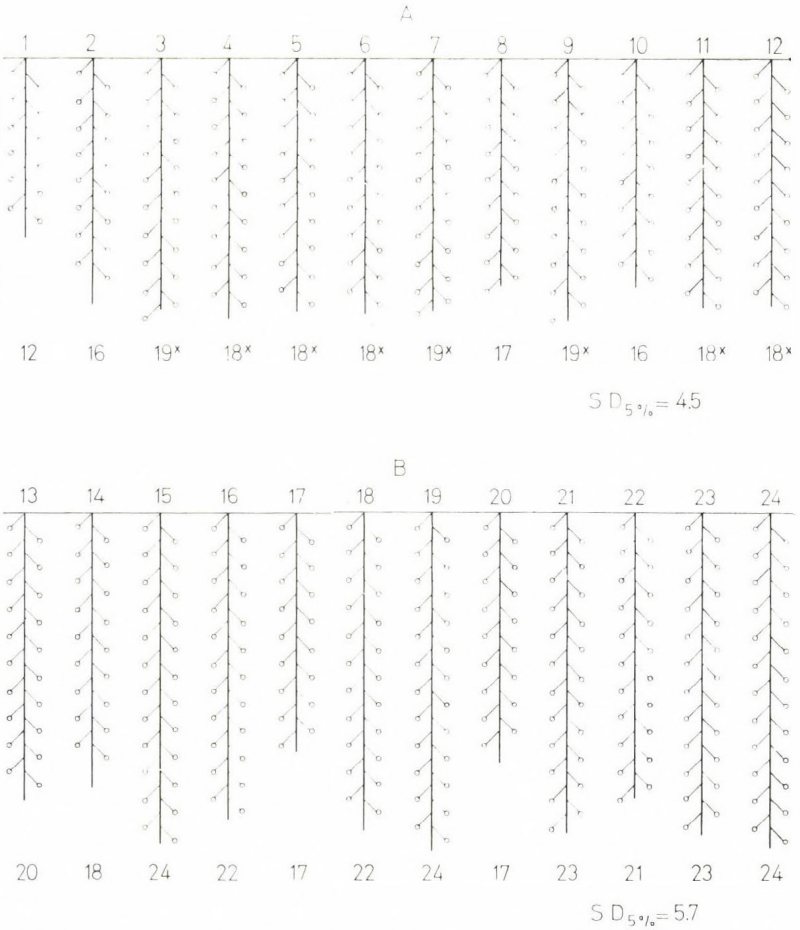


Fig. 2. The formation of root nodules on the effect of treatment. Treatments: The same as in Fig. 1. Number of nodules. *A)* Uninoculated series $SD_{5\%} = 4.5$. *B)* Inoculated series $SD_{5\%} = 5.7$

creased the P and K content. These effects were indistinct in the case of the inoculated series.

In the investigations conducted in acidic forest soil, the protein-N content of plants increased on the effect of some treatments (Fig. 1). This increase was significant at 0.1% level only. Mo-treatment did not increase the protein content of investigated plants. In the experiments carried out in calciferous soil the plants of the four replications were not examined separately, so statistical analysis could not be done but the protein-increasing effect of the treatments could be observed in this case too, which was 1—13% compared with the control.

All treatments favourably influenced the formation of the number of root nodules (Fig. 2). As regards the increase of number of root nodules, the differences proved significant (at $P_{5\%}$ level) in all treatments with the exception of B, B + T and Mn + T ones.

The effect of Ceresan and Thiuram on four strains of *Rhizobium leguminosarum* was studied in laboratory tests too. Both examined fungicides inhibited the development of all strains (Table 3). But it could be established that D_1 strain was relatively the most resistant to both fungicides. The strain TA 101 to the same extent as the D_1 was also relatively resistant to Thiuram.

Ceresan had about the same degree of inhibitory effect on the other examined three strains as Thiuram on the other two strains.

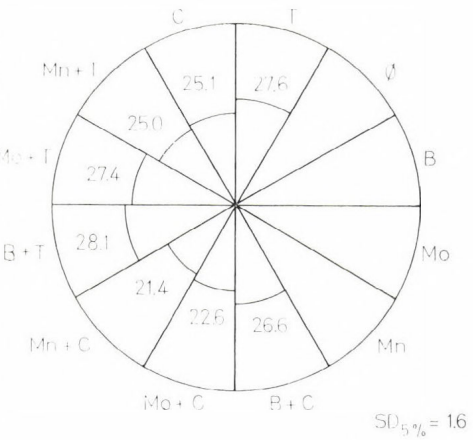


Fig. 3. The inhibitory effect of microelements, fungicides and combinations of treated seeds on the growth of *Rh. leguminosarum*. SU 391. + Treatments: as in the case of Fig. 1. ++ Inhibitory zones in mm. $SD_{5\%} = 1.6$

The effect of different treatments on the SU 391 strain of *Rh. leguminosarum* was also tested, using seeds treated with the listed materials (Fig. 3). It was established that neither microelement treatment nor *Rhizobium* inoculation had an inhibitory effect. But the treatments of fungicides and their combinations with microelements all inhibited the growth of SU 391 strain. This inhibitory effect proved significant in every case though the different size and shape of the treated vetch seeds as well as their position on the surface of the medium, did not give nearly so precise data as the results received with the filter paper discs containing the investigated materials (Kecskés and Vincent 1969a). Because of this, the scatter was large enough. The inhibitory effect of Ceresan was significantly decreased by the two microelements Mo and Mn (at $P_{5\%}$ level).

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Table 3

Inhibitory effect of Ceresan and Thiuram on the different strains of Rhizobium leguminosarum

Strains	Fungicides	
	Ceresan	Thiuram
	Inhibitory zone Ø in mm	
D 1	23	18
BO	41	36
13 B	48	35
TA 101	42	18

SUMMARY

Separated and combined treatments of microelements (Molibden, Bor, Manganase and Fungicides (tetramethyl-thiuram-disulphide; phenyl-mercury-acetate and ethoxy-ethyl-mercury-silicate) were carried out on vetch *(Vicia sativa L.)* and the rhizobia as well as their symbiosis. In laboratory tests both fungicides inhibited the growth of all the investigated strains of *Rhizobium leguminosarum*.

In pot experiments the microelements and fungicides applied separately and in combination increased the dry matter yield and protein content of vetch grown in sandy soil. They also favourably influenced the root nodule formation of vetch that is its symbiosis with rhizobia. Among the treatments, the combined treatments of Ceresan and microelements had the best effect on the investigated factors.

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THE INFLUENCE OF UREASE AND S-TRIAZINES ON RHIZOBIA AND GRAIN YIELD OF *LUPINUS LUTEUS L.*

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The formation of the grain yield of the yellow flower sweet lupin is influenced besides the climatic factors, by the extent of weediness (Ubrizsy 1962). Mechanical weeding is hardly carried out because of the density of sowing. Chemical weed control is consequently one of the basic important conditions of successful and profitable seed-growing. But chemical weed control, as pointed out by Taylor (1961) Ubrizsy (1961), Kolosova (1962), Gimesi (1965) and others is complicated by the extraordinary sensitivity of lupin.

On the effect of some herbicides, the plants are retarded in their growth and development. These symptoms are quite similar to the cases when root nodulation of lupin did not take place or was inhibited for some reason.

In the course of our investigations we established — in accordance with other authors — the importance of studying the effect of pesticides on rhizobia. So, e.g. there was no herbicide among the 106 agents applied by us (Kecskés 1970) in excess to the media which did not inhibit growth of any of the investigated 26 representatives of 6 species of *Rhizobium* genus. From our experiments carried out in field with fungicides we stated the necessity of studying the effect of the these chemicals on the root nodule formation (Kecskés and Vincent 1969a) and dry matter yield of legume plants (Kecskés and Vincent 1969b, 1970).

Keeping in mind all of these, we aimed to select such a herbicide which beside its good weed control effect did not damage either the lupin rhizobia, or the symbiosis of the plant and rhizobia.

MATERIAL AND METHODS

The laboratory investigations were conducted in the Research Institute of Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, the field trials were carried out in Gyulatanya on slightly acidic brown forest soil of the Plant Breeding Station of the Agricultural Experimental Institute of Nyírség.

The following herbicides were studied in both laboratory and field trials (Table 1).

For laboratory tests Yeast Mannitol Agar medium (YMA: Kecskés and Vincent 1969a I) was used. The listed herbicides were applied with filter paper discs to this medium in excess.

Test organisms: 26 strains of *Rhizobium lupini* and — for comparison — other five species of *Rhizobium* genus. Field experiments were con-

Table 1

I. UREAS

1. KLOBEN (60% NEBURON)
   N-(3,4 Dichloro-phenyl)-N'-methyl-N'-butyl-carbamide
2. ARESIN (MONOLINURON)
   N-(4-Chloro-phenyl)-N'-methoxyl-N'-methyl-carbamide

II. MERCAPTO-S-TRIAZINES

3. A 1114 (PROMETRYNE)
   2-methyl-thio-4,6,-bis (iso-propyl-amino)-1,3,5-triazine
4. A 1803 (PROMETRYNE + SIMAZINE)
   PROMETRYNE + 2-chloro-4,6-bis-(ethylamino)-1,3,5-triazine

ducted with three factors; the split-split-plot arrangement and quadruplications were used. The size of one plot was 30 m². Gyulatanya 784 of yellow flower sweet lupin *(Lupinus luteus L.)* was used as the test plant.

Factor A: the times of herbicide spraying.
A_1 — between sowing-rising
A_2 — in the 5-leaf developing phase of lupin
A_3 -- in the 10-leaf developing phase of lupin
Factor B: the different concentrations of herbicides
B_1 — 2.0 kg/ha in the case of Kloben 4,5 kg/ha
B_2 — 2,9 kg^{8}ha in the case of Kloben 5.2 kg/ha
B_3 — 3.5 kg/ha in the case of Kloben 5.9 kg/ha
Factor C: Treatments
C_1 — hoed control
C_2 — unhoed Control
C_3—C_6 — different herbicides

The following were determined for the lupin (a) and for the weeds (b) during the growth season on 1 m² permanent sampling places

a) the number of plants at rising, the root nodule formation at flowering the number of plants and the amount of grain yield at ripening

b) The number of weeds per species and the whole weight of weeds in the permanent sampling places divided into four parts three-times: 10 days after spraying, at the end of flowering and 20 days after ripening.

RESULTS

Data of laboratory investigations are demonstrated in Table 2. Inhibitory zone values received from the average of the data of all the 26 strains, and strains belonging to different species, uniformly show that among the stu-

Table 2

Inhibitory zone of herbicides on agar plate

Rhizobia / Herbicides	*leguminosarum sp.*	*phaseoli sp.*	*trifolii sp.*	*lupini sp.*	*japonicum sp.*	*meliloti sp.*	Average of 26 strains
Kloben	7.6	8.8	6.0	0.0	22.2	3.7	8.94
Camparol 1803	13.2	9.4	8.6	14.0	20.3	7.4	10.95
A 1114	24.0	23.6	23.0	24.0	24.8	17.4	24.33
Aresin	33.9	33.4	38.5	12.0	50.4	28.6	35.64

Fig. 1. Roots (with nodules) of 10 plants each taken out by random sampling from the hoed control Kloben and Aresin treated plots at the stand spraying in 10 leaf stage

died herbicides the Kloben inhibited the least the growth of rhizobia — it was followed in increasing inhibitory order by Camparol 1803 and the A 1114; Aresin proved the significantly strongest inhibitory herbicide.

Similar results were obtained in the course of detailed examination of the root nodulation forms. No sample was found which did not have nodules on its root. Definite delayed root nodulation forms could not be observed. But one has to consider, that because of the experiments which have been carried out with lupin over a few decades in the soils of the Plant Breeding Station of Gyulatanya they are rich in effective rhizobia. Inspite of them, there were delineating differences in the development and mass of root nodules, in Fig. 1. The roots of 10 plants, each taken out by random

Fig. 2. Root samples taken out randomly at spraying in the 5 leaf stage of plants

sampling from the hoed control Kloben and Aresin treated plots at the stand spraying in the 10 leaf stage can be seen. As it is evident Kloben compared to the hoed control did not inhibit unfavourably the root and root nodule formation too. However, in the effect of Aresin belonging also to the ureas, the development of roots and nodules was obvious. The root samples taken out at spraying in the 5 leaf stage of plants demonstrated (Fig. 2) also that there were no significant differences among the plants of the plots of hoed, unhoed, control and treated with Kloben. But the roots and nodules originating from Aresin, A 1114 and Camparol 1803 treatments showed defined differences compared with the earlier ones. The same trend was observed in the case of the summarized data of the root nodule weights of 10 plants each taken from every plot of all treatments (Table 3).

Table 3

Weight of lupin roots and nodules in different treatments

Treatments	Weight of root of 10 plants in gram
1 hoed Ø	37.5
2 Ø	36.8
3 Kloben	35.0
4 A 1114	30.5
5 A 1803	27.3
6 Aresin	22.3
$SD_{5\%}$	7.31

Table 4

Amount of lupin grain yield kg/plot

Year	Treatment	Times of spraying		
		A_1	A_2	A_3
1968	Aresin	1.35	0.86	0.67
	A 1114	1.30	0.73	0.70
	A 1803	1.50	0.64	0.80
	Kloben	1.57	1.00	1.07
	Hoed	1.54	1.10	1.11
	Without hoeing	1.24	0.97	1.14
		$SD_{5\%}$ among spray times among treatments		
1969	Aresin	2.22	1.95	1.61
	A 1114	2.48	0.86	0.56
	A 1803	2.94	0.85	1.22
	Kloben	2.52	2.36	2.08
	Hoed	2.82	2.89	2.95
	Without hoeing	1.71	1.95	2.22
		$SD_{5\%}$ among spray times among treatments		

Analysing the effect of herbicides on grain yield we established that the different applied doses did not give significant differences. Among the herbicides studied by us, only Kloben applied pre- and postemergently did not decrease significantly the grain yield of lupin (Table 4). The grain yield was significantly similar only in two cases in Kloben treatments compared with the hoed control. Among the other herbicides, three applied in three different doses at three different times only the Camparol 1803 sprayed preemergently did not decrease significantly the grain yield of our test plant. Aresin (Fig. 3) used for stand spraying as opposed to the Kloben (Fig. 4) scorched the lupin and as a consequence of this, the plant stand spaced out.

As regards weed control effect of herbicides (Table 4) it turned out that the weight of weeds in the plots treated with Kloben was significantly more than on the hoed, control plots in only one case. The weed control effect of Kloben was nearly as good as the mechanical weed control (Table 5). The other three herbicides gave different results yearly and at the different spraying times.

Table 5

The weight of weeds 0.25 m²/g

Year	Treatments	Times of spraying		
		A_1	A_2	A_3
1968	Aresin	339.5	299.1	216.1
	A 1114	371.7	122.0	122.5
	A 1803	303.1	152.5	208.0
	Kloben	312.5	185.6	188.2
	Hoed	286.8	290.4	217.3
	Without hoeing	349.0	292.7	274.6
		$SD_{5\%}$ among spray times among treatments		
1969	Aresin	262.3	369.7	483.8
	A 1114	271.2	478.6	418.2
	A 1803	240.5	339.9	271.5
	Kloben	257.9	207.6	270.1
	Hoed	215.2	199.9	151.0
	Without hoeing	326.5	366.3	459.9
		$SD_{5\%}$ among spray times among treatments		

The summarized data are demonstrated in Fig. 5. It is very interesting that the data of the earlier laboratory investigations confirmed unanimously the results of field experiments, and the data of nodule formations and grain yield of lupin supported it markedly. It is obvious from this Table that among the investigated herbicides one could reach the highest yield of lupin with the Kloben treatment, the weed control effect is favourable too and the growth and activity of rhizobia is not inhibited by it.

Fig. 3. The negative effect of Aresin spraying on lupin stand

Fig. 4. The favourable effect of Kloben spraying on lupin stand

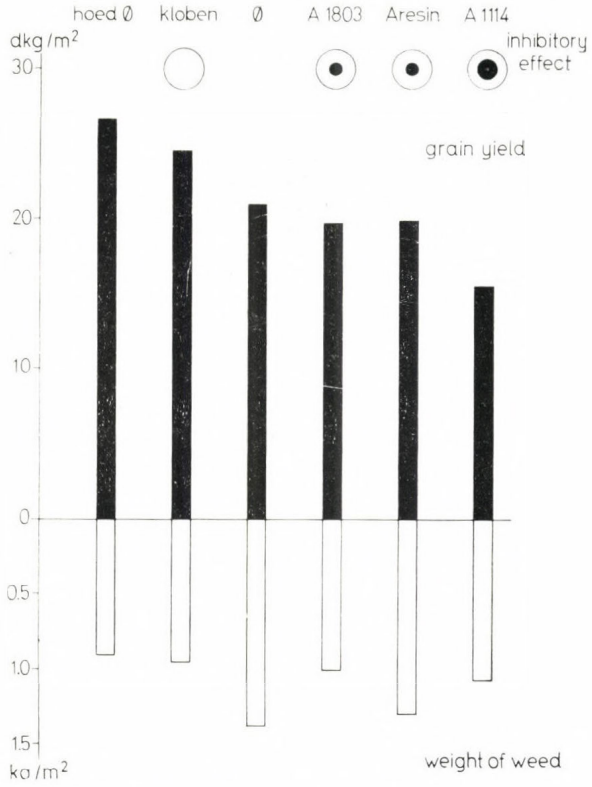

Fig. 5. A survey of the data received in the course of herbicide treatments: 1) inhibitory effect on rhizobia; 2) grain yield of lupin; 3) weight of weed

SUMMARY

On the basis of the laboratory investigations and two years' field trials it can be established that from the studied herbicides — Aresin A 1114, Camparol 1803, and Kloben, the Kloben was that which:

1. Hardly or not at all inhibited the growth of rhizobia.
2. Did not influence disadvantageously the root nodulation, the symbiosis of yellow flower sweet lupin and rhizobia.
3. Did not decrease significantly the grain yield of yellow flower sweet lupin — sprayed neither preemergently nor postemergently
4. Had a weed control effect which was nearly as good as the mechanical (hand hoeing) weed control.

On the basis of the concordant results of laboratory and field experiments on the yellow flower sweet lupin in the main growing regions of Hungary, Kloben is recommended by us in slightly acidic brown forest soil — for the weed control of this lupin species.

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EFFECT OF CERTAIN HERBICIDES ON GROWTH OF NITROGEN-FIXING ALGAE AND RICE PLANTS

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Recently, great attention has been paid to the beneficial effect of inoculating rice fields with nitrogen fixing blue—green algae (Allen 1956, Watanabe 1962, and Watanabe et al. 1951). In UAR, inoculation with alga *Tolypothrix tenuis* greatly increased yield of rice grains and straw as well as soil nitrogen; where nitrogen balance was found to be in the positive side. Algal inoculation could substitute, at least, half the amount of the added nitrogen (El-Nawawy et al. 1958, Abou El-Fadl et al. 1964, 1967 and El-Sherbini 1969).

Control of weeds in rice plantation is however, becoming a general practice. Hence, it was found of interest to study the effect of certain herbicides namely, Eptam 6-E, Stam F-34, Ordram, and Trifluralin on nitrogen fixation and growth of algae namely, *Tolypothrix tenuis* and *Calothrix brevissima*, as well as growth of rice plants. Hamdi et al. (1970) in this respect showed that dry weight and nitrogen content of alga *Tolypothrix tenuis* were reduced in the presence of Ordram, Trifluralin, 2,4-D and Stam added either at the begining or 10 days after growth initiation; where Stam was toxic to algal growth and chlorophyll synthesis. They recommended the application of herbicides to rice fields after the algal inoculation. Stam however, should be avoided at all if the alga is to be used.

MATERIAL AND METHODS

Algae *Tolypothrix tenuis* and *Calothrix brevissima* were propagated in 500 ml conical flasks containing 100 ml of sterilized nitrogen-free medium (Watanabe et al. 1951).

A laboratory experiment was carried out in 150 ml conical flasks containing 25 ml of the afore-mentioned medium. Aqueous solutions of the tested herbicides namely, Eptam 6-E (Ethyl-di-n-propylthiol-carbonate), Stam F-34 (3,4-dichloropropion-anilide), Ordram (s-ethylhexahydro-1-M-azepine-1-carbothionate), and Trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N-di-propyl-p-toluidine), were added in the following concentrations prepared on the active ingredient basis: 0.01, 0.1, 1.0 and 10.0 and 100.0 ppm. Flasks prepared in 4 replicates were inoculated with a loopful of algal inoculum 3 weeks old, and kept under direct and undirect sunlight. After 8 weeks, algal growth was filtered, dried (80 °C overnight), weighed and its nitrogen content was determined.

For the greenhouse experiment, earthenware pots 25 cm in diameter were used. A fertile loamy soil was obtained from The Botanical Department, Ministry of Agriculture at Giza. The soil was air dried, ground and sieved in 2 mm sieve. Its chemical analysis was as follows: Organic matter 1.03%, Total N 0.13%, Soluble N 175 ppm, T.S.S. 0.18% and pH 7.8.

Each pot received 5 kg of the soil thoroughly mixed with 1.0 g superphosphate. Rice seeds (Nahda variety) were planted at the rate of 10 per pot; where moisture was kept at 60% of w.h.c. Seedlings were thinned 2 weeks later to keep 6 per pot. Algal inoculation was done after 4 weeks from planting; where each pot received 1 ml of the algal suspension (dry matter 0.03 g). Water was added daily to compensate that lost by evaporation and to maintain the flooding stage.

Herbicides were added in aqueous solutions at the recommended field dose (Zahran 1970) as follows:

A — 1 week before inoculation,

B — just after inoculation,

C — 1 week after inoculation.

Four replicates were prepared for each treatment; where pots were completely randomized in the greenhouse. After 2 months plants were cut at the soil surface, dried (80 °C, overnight), weighed and its nitrogen content was determined. Semi-micro Kjeldahl method was used for nitrogen determination according to Jackson (1958).

RESULTS AND DISCUSSION

As shown in Table 1, algal growth and nitrogen fixation were inhibited in the presence of 0.1 ppm of the tested herbicides. Rate of inhibition was not the same with the tested herbicides and the alga. Lowest dose namely 0.01 ppm, however, slightly stimulated algal growth as well as nitrogen fixation. No growth was recorded in the presence of 100 ppm. of Eptam, Stam and Trifluralin.

For *Tolypothrix tenuis*, Eptam, Stam and Trifluralin were found to be the most toxic herbicides; where no growth was recorded in the presence of 10 and 100 ppm. Stam however, showed less toxicity on the algal growth and rate of nitrogen fixation; were 145.51, 116.44, 66.54 and 161.62 mg nitrogen were fixed, in the presence of 0.1 ppm of Stam, Trifluralin, Eptam and control respectively. Ordram however, was found to be the least toxic herbicide; where growth was recorded even in the presence of 100 ppm.

Similar results were recorded for *C. brevissima*. However, this alga showed more sensitivity to Ordram and less sensitivity to Trifluralin. 80.12, 78.49, 51.64, 44.64 and 82.95 mg nitrogen were recorded in the presence of Trifluralin, Stam, Ordram, Eptam and control respectively.

In this respect, it must be stated that *T. tenuis* showed more growth, and was more efficient in nitrogen fixation than *C. brevissima*; when grown in the nitrogen-free medium of Watanabe.

Growth of rice plants (Table 2) was greatly affected by the application of herbicides. The benefit from algal inoculation seemed to be greatly affected by the time of application. Less growth was recorded as herbicides were applied either with inoculation or before it. Application of herbicides later

Table 1

Effect of herbicides on algal growth and nitrogen fixation

Herbicides, ppm		*Tolypothrix tenuis*			*Calothrix brevissima*		
		Algal dry wt., g/l	Total N content		Algal dry wt., g/l	Total N content	
			%	mg		%	mg
Eptam 6—E	0.01	2.760	7.14	197.06	2.028	4.36	88.42
	0.10	1.492	4.46	66.54	1.043	4.28	44.64
	1.00	1.425	4.12	58.71	0.094	2.78	26.13
	10.00	—	—	—	—	—	—
	100.00	—	—	—	—	—	—
Stam F—34	0.01	2.962	7.14	211.49	2.013	4.28	86.16
	0.10	2.038	7.14	145.51	1.817	4.32	78.49
	1.00	0.916	5.42	49.65	1.642	4.32	70.93
	10.00	—	—	—	—	—	—
	100.00	—	—	—	—	—	—
Ordram	0.01	2.285	7.64	174.57	1.423	4.62	65.74
	0.10	2.137	7.16	153.01	1.291	4.00	51.64
	1.00	2.153	7.08	152.43	0.214	2.06	4.41
	10.00	1.292	6.14	79.33	—	—	—
	100.00	0.148	4.12	6.10	—	—	—
Trifluralin	0.01	2.137	7.64	163.27	1.654	4.62	76.41
	0.10	2.051	7.14	116.44	1.872	4.28	80.12
	1.00	0.680	2.48	16.86	0.681	2.14	14.57
	10.00	—	—	—	0.442	2.48	10.96
	100.00	—	—	—	—	—	—
Control		2.278	7.4	161.62	1.938	4.28	82.95

after inoculation showed more growth and nitrogen uptake. Herbicides applied before inoculation may have lost their toxic effect as they were decomposed by microflora or adsorbed on the clay mineral complex in the soil. The established alga when applied to the soil before application of herbicides, could resist their toxic effect. The liberated nitrogen due to the decomposition of algal cells could be easily absorbed and utilized by plants.

Eptam and Trifluralin were found to be the most toxic herbicides for plant growth and nitrogen uptake. Stam and Ordram showed no toxicity when added one week after inoculation.

Hence, it can be concluded that Stam and Ordram could be applied to rice plantations, after algal inoculation.

SUMMARY

Nitrogen fixation and growth of algae *Tolypothrix tenuis* and *Calothrix brevissima* were inhibited in the presence of herbicides Eptam 6-E, Stam F-34, Ordram and Trifluralin. Lowest concentration namely 0.01 ppm. slightly increased rate of fixation and growth of algae.

Table 2

Effect of herbicides and inoculation with alga Tolypothrix tenuis on dry matter yield of rice plants and their nitrogen contents

Herbicides		Dry matter yield g/pot	Total N content		
			%	mg	Control = 100
Eptam 6—E	A	1.42	1.40	12.88	14
	B	0.92	1.32	12.14	12
	C	1.28	1.32	16.89	18
Stam F—34	A	5.88	1.20	70.86	78
	B	6.08	1.18	71.74	79
	C	7.34	1.24	91.02	101
Ordram	A	6.85	1.08	73.98	82
	B	6.40	1.15	73.60	81
	C	7.36	1.18	86.85	96
Trifluralin	A	3.21	1.15	36.91	41
	B	3.46	1.15	39.79	44
	C	4.59	1.25	57.37	63
Control	inoculated	7.25	1.24	89.90	100
	uninoculated	6.42	1.15	73.83	82
L. S. D. 05		0.40			

A: Herbicides applied 1 week before inoculation.
B: Herbicides applied just after inoculation.
C: Herbicides applied 1 week after inoculation.

Eptam 6-E was found to be the most toxic herbicide for nitrogen fixation, growth of rice plants and nitrogen uptake. This was followed by Trifluralin which showed lowest toxicity. Stam F-34 and Ordram however, showed no toxicity on growth of rice plants and nitrogen uptake.

Herbicides should be added later after the establishment of alga in the soil; where plants produced more dry matter yield and nitrogen content.

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