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SOIL MICROBIOLOGY IN THE USSR

Edited by

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P R E F A C E

The expansion of the sowing area and, in particular, of that under technical and leguminous crops, the introduction of novel leguminous crops, the chemization of agriculture and the development of the fertilizer industry bound with it have confronted Soviet agricultural science, directly after the Revolution, with a whole series of new and essential problems.

These problems, naturally, stimulated to a great extent the development of soil microbiology.

The latter had to aid in finding ways for a more complete utilization of fertilizers by their conversion into a form assimilable to plants, by means of the activity of soil microorganisms.

From this point of view, the task of soil microbiology may be summarized as the following three fundamental problems:

1. To provide a microbiological picture of soils, and to ascertain their preparedness for different crops and their requirements in various fertilizers.

2. To provide directions for the rationalization of storing and of the preparation of organic fertilizers, such as stable manure, peat and others.

3. To provide cultures of nodule bacteria for the nitrogenization of new lands.

The scientific microbiological work which had begun throughout all of the Union was distributed at the outset of the Second Five-Year Plan between a whole series of new laboratories in the following way.

The head institute along the line of the People's Commissariat for Agriculture is the All-Union Institute of Agricultural Microbiology of the Lenin Academy of Agricultural Sciences (Leningrad, Gerzen Street, 42) founded by S. P. Kostytshev (Moscow branch — Agro-biological station, director A. F. Wojtkiewicz). In contact with the latter are working microbiological groups of other Institutes of the Lenin Academy; the All-Union Institute of Manures and Agricultural Pedology (founded in 1931) — Moscow, Petrovskoie-Rasumovskoie, Academic passage № 1; branches in Leningrad, Tiflis, Tashkent and others. The Ukrainian Institute of Agricultural Chemistry and Pedology (Kiev); the Peat Institute (Moscow); the Ukrainian Peat Institute (Kiev); the Institute of Flax (Torzhok) and the subordinated zonal, regional and other experimental stations.

Along the line of the fertilizer industry, subordinated to the People's Commissariat for Heavy Industry, scientific work is centered in the Scientific Institute on Fertilizers (N. I. U.) (Moscow, Pizhewsky 7) founded by J. W. Samoilov in 1917. This Institute possesses a microbiological division (chief E. E. Uspensky).

Along the line of the People's Supply Commissariat the study of soil microbiology is carried on in its Institutes: Central (Moscow) and Ukrainian (Kiev), in the Institutes of Sugar Industry, in the Institutes of Tobacco culture (Krasnodar), of Makhorka culture (Kiev). Further work is carried on in the Institute of Rubber (Moscow) and Cotton (Tashkent) of the People's Commissariat for Light Industry, and in others.

Besides that, the corresponding chairs of Universities and Academies of Sciences give much attention to soil microbiology.

At the All-Union Conferences, in 1932 and 1933, All-Union joint programmes of scientific research work, providing for its distribution among individual institutions, have been elaborated in accordance with the main problems enumerated above.

Besides this, in respect to the first problem, according to local peculiarities of the different regions, the study of important special problems, connected with the culture of cotton and of other technical crops, as well as with the shifting of the grain crops eastwards had to be planned. These were, first of all, the problem of the microbiology of soils in irrigated regions, and that of the microbiology of salty soils.

As we see, the most essential problems of soil microbiology in connection with Socialist construction, fit in very well into the scheme of research elaborated by the III Commission of the International Society of Soil Science for the Conference at Copenhagen in 1933. The All-Union Conference in Leningrad (24—27 December, 1932) therefore approved of the above-mentioned programme for the Copenhagen Conference and designed reports which are to be presented to it by the USSR.

E. Uspensky

SOIL MICROBIOLOGY IN THE USSR (1917—1932)

E. E. USPENSKY

Moscow, USSR

Introduction

I. Object of investigations

Fifteen years ago soil microbiology as such did hardly exist in our country. The first aim of the present review is, therefore, to note and outline the essential moments of the origin and formation of our science, further on — its coming forth into the wide field of activity with the beginning of the first five-year plan of socialist construction (1928) and, finally, its prospects for the future deriving from problems of the second five-year period, as well as from the achievements of the West, — as a groundwork for the new plan.

At the same time it would be wrong to begin, as though soil microbiology had arisen from nought and only in connection with new demands. Just on the contrary it has received a very sound inheritance from different quarters and, first of all, from its own source—the general microbiology of Winogradsky—Omeliansky, and the Russian plant physiology of Timiriazeff—Palladin. It is necessary, — for making it actually clear in what way soil microbiology has arisen and in how far it has progressed during 15 years — to dwell briefly on its initial points and, first of all, to sum up what has been taken from this inheritance by Soviet science after the October Revolution.

II. What has Soviet microbiology inherited from the preceding period

Microbiology is as yet a young science. In spite of the discovery of the microscopical world by Kircher and Leeuwenhoek, so far back as in the XVIIth century, our progress in its study was very slow, until a sufficient perfection had been reached in the construction of the microscope (de-Amici, water immersion, 1848; Stephenson, oil immersion, 1873); until chemistry and biochemistry had begun to take ground,

until the powerful development of surgery and medical science in general and of a series of industries (canned food, public water supply, alcohol and beer production, etc.) confronted microbiology.

Only industrial Europe, already firmly footed towards the second half of the XIXth century, presented a sufficient basis for the rapid growth of the new science, Louis Pasteur's works playing the leading part in its development.

Pasteur's works, as well as those of his disciples, found a most vivid response in Russia. S. Winogradsky in his very first works attacked problems of a general theoretical importance. It suffices to bring to mind the discovery of the chemosynthesis by Winogradsky, the establishment of the elective method, the thorough study of the physiology of sulfur oxidizers, of nitrifying bacteria, of the first freeliving nitrogen fixing microorganisms, of iron oxidizers, as well as certain works of his disciples (Omeliansky, decomposition of cellulose; V. Friebes, pectin fermentation, and others) — to show that a new road had been struck by science, which solved, by specific methods, cardinal problems of general microbiology.

Thus, supplementing the carbon cycle of transformation, justly considered to be the greatest discovery of physiology, the harmonious picture was outlined of the cycle of another, most important element — of nitrogen, and the way shown for establishing a series of other cycles. Besides, such cycles of transformation appeared in a new light, chemosynthesis having appeared on the scene.

Further on, in 1909, W. L. Omeliansky summed up all these works, by editing his manual of an exclusively great importance, — a treatise on the cycles of the transformation of substances in nature and of the rôle of microbes in these processes.

The following facts are to be noted. Winogradsky solved transformation cycle problems that had been brought forward and partly disclosed by Western agrochemistry. Schloesing and Münz who had established the fact of nitrification under ordinary laboratory conditions, Berthelot in his tests on nitrogen assimilation, were, certainly, direct predecessors of Winogradsky. Yet, the theoretical solution of Western problems was advanced by representatives of a country far from being able to put into practice the improvements of scientific agronomy. Before the revolution no economical basis had existed in Russia for any noticeable application of artificial fertilizers.

Winogradsky's works could not attain that merging with practical life, which is so characteristic of microbiology (Pasteur, Hansen, and others) from its very birth.

In fact, the Russian scientific pre-revolutionary agronomics did not adopt Winogradsky-Omeliansky's ideas¹.

The forward Russian school of D. N. Prjanschnikov may be taken as an example. When it came to confront microbiology, the only thing it valued in it was the method of pure cultures, allowing to exclude the uncalled for influences of microbes on the physiology of nutrition.

The agrochemist, having lost his way in some problem and not finding any issue, had recourse to «biological activity» and, citing Winogradsky's highly gifted works, found a suitable «explanation».

When Omeliansky's manual made its appearance and the rising generation began studying it, a wonderful picture might have been observed: every experimental station studied «nitrification», whole systems for a natural restoration of fertility based on the work of microorganisms were being constructed—but no culture of nitrifiers could be found anywhere in Russia. It proved, when being checked, that the «investigators» in nitrification had, perhaps, even never seen these cultures. All work was confined to the determination of nitrates and nitrites in soil. Nothing else had remained of Winogradsky in the Russian pre-revolutionary agronomic activity.

It is necessary to note, that all essential Winogradsky's works of that trend were made in the XIXth century, so that this trend comes to be almost extinguished in the beginning of the XXth century.

It would be wrong, however, to consider Winogradsky's and Omeliansky's microbiology, as the sole predecessor of the Soviet soil microbiology. As I had noted at the very beginning, there can be no progress of microbiology without biochemistry. When, at the beginning of the XXth century, a deep depression in the domain of plant physiology and in the physiological trend in agrochemistry was caused in Europe by a general reaction and special emphasis on colonies, chemistry continued its flourishing development. Biochemistry as well developed with exclusive speed. K. A. Timiriazeff's most talented disciples took especially to one of his mottoes: «chemist — and botanist at the same time». The majority of Russian physiological schools began working in that direction. Palladin's school attained an exclusive success. Representatives of that school constantly utilized microorganisms as subject of their researches on the chemical aspect of all essential vital processes. This school exerted an exclusive influence on the development of Soviet soil microbiology.

¹ Microbiology was found to have deeply penetrated but in certain works of the P. S. Kossowitsch's and A. I. Nabokikh's laboratories.

and it is on its basis that the Microbiological Institute of the All-Union Lenin Academy of Agronomical Sciences arose, organized by S. P. Kostytschev¹.

Unfortunately, it had so turned historically that Leningrad, the essential centre of the prerevolutionary microbiology, had never been a centre for the study of the application of fertilizers. It was difficult to advance without removing these discrepancies, and it was the lot of the revolution to solve this problem.

Palladin's school, besides its neglect of various problems maturing in the agronomic periphery, had narrowed the very biochemical way of tackling problems characteristic of C. A. Timiriazeff.

Terminating the Croonian lecture (p. 459) Timiriazeff says:² «From this point of view this field of inquiry on the cosmical function of the green plant is a kind of borderland between two of the greatest generalisations of the last century, associated with the names of Lord Kelvin and Charles Darwin — the principle of the dispersion of energy and the principle of the struggle for life».

This C. A. Timiriazeff's thesis remained forgotten. Actually, these greatest achievements of the second half of the XIXth century were not taken into account by biochemists. The biochemical school in soil microbiology found itself isolated not only from the essential measures taken for the regulation of the biodynamics of soil, fertilizers, etc., but even from the study of the essential principles of dynamics.

Besides the trends just examined, there had existed in Russia before the revolution one trend in microbiology, which might be called botanical, its greatest representatives being G. A. Nason and A. P. Artari. We do not find in it the conception of a biochemical cycle of substances as the impellent spring of its investigations, nor a physiology having a special biochemical bias. But we find a harmonious linking of biological moments with those adjacent, coming into contact with them. Everything is studied on a background, formed by the cycle of the organism's development taking harmoniously into account the physiological functions and morphological changes in connection with the peculiar conditions of the organisms. The ideas of evolution and adaptability are the essential active principles.

As usual under the conditions of tsarism, the chief representatives of the mentioned trend were kept at some distance from the university chairs. Only just before the revolution G. A. Nason succeeded to organize the review «Microbiology», his trend being vividly expressed in it. After the revolution it served as

¹ Very near to this trend were also W. K. Zalesky's and W. S. Butkewitsch's laboratories.

² Timiriazeff, C. A. The Cosmical Function of the Green Plant. Proceed. of the Royal Society of London, LXXII, 424—461 (1904).

basis for the work of the microbiological laboratory of the Academy of Sciences.

A. P. Artari, in spite of his leading works of world wide importance on physiology and on pure cultures of algae, remained lecturer without a laboratory in the Moscow University almost up to the October Revolution.

We have shown above that the profoundly theoretical trend of Winogradsky-Omeliansky had become markedly weaker at the beginning of the XXth century. Yet, generally speaking, the decline in microbiology was lesser than, for instance, in plant physiology. The trend we had called botanical gradually strengthened, not to mention the biochemical trend just then being developed. Besides, direct farming practice was beginning to rouse microbiology to life.

In the line of agriculture microbiology was attracting the attention of practical workers, by the possibilities of utilization of the culture of root nodule bacteria. Other branches of agriculture were also in need of microbiologists, such as dairy - produce, struggle against rodents and others. In 1894 the Society for Acclimatization founded a special Agrobacteriological Station, which in 1897 began the publication of its review «The Messenger of the Agrobacteriological Station». A similar laboratory was founded almost at the same time by the Ministry for Agriculture.

Yet, even at that time, S. A. Severin had written of the latter that «it had been inaugurated so far back as in 1891, its tasks being very modest», and that «its staff together with a widened programme of activity had been definitely established but in 1906». As to the Agrobacteriological laboratory, in spite of its essential problems having been clearly formulated by S. A. Severin (its director from 1894 till 1914), in practice much of its attention had to be given to current small problems — analyses and making preparations — in order to earn its means of subsistence.

The Moscow Agrobacteriological Laboratory conducted by A. F. Wojtkiewicz, contrived to utilize the small pre-revolutionary scientific capital, in order to attract new forces from the very first years after the revolution, and to start an essential work on soil microbiology.

We see, on brief examination of the prerevolutionary microbiology and its agronomical part, that its great achievements and many exclusively valuable workers remained outside Russian actuality. This finds its expression in the following facts.

1. Microbiology did not proceed from problems of Russian agronomics. The more accessible at that time ways were elucidated the least. Thus, we may note that, in spite of the general high level of microbiology, disproportionately little was done towards the study of root nodule bacteria, i. e. of problems of green fertilizers, of crop rotation, of fodder grass, and so on.

The problem of manure, as a matter of fact, had not been touched. But the very first steps had been made in the problems of the utilization of phosphorites. Nothing had been said on microbiology in connection with liming, although all the enumerated measures should have been the first real steps, production of mineral fertilizers being absent.

2. Scientific work and its immediate approach to agronomics had not been organized¹. With Winogradsky having left that work, the whole of the movement started by him came to a standstill. Nadson's and Artari's works did not get any direct support.

3. Actually, the organizing moment — Omeliansky's manual — remained for the elder generation well-nigh «the voice of one crying in the wilderness». It only gave them good «explanations» for finding an issue out of difficult circumstances. In practice microbiology without microbes was prevailing.

4. The agro-bacteriologic periphery was only arising and, mainly, solved narrow utilitarian current problems.

5. Except the organized school of plant physiologists-biochemists of V. I. Palladin and some others of a lesser eminence, no cadres having a deeply theoretical grounding were being prepared hardly anywhere. There existed but one university chair of general microbiology and that with a medical bias (Bardach) in Odessa. Courses for lecturers without laboratories were to be found only at universities in capitals, lectures not being given even every year.

6. Chairs of agricultural bacteriology and physiology of plants existed at higher agricultural schools. Yet, taking into consideration that the higher agricultural school had no right (according to the pre-revolutionary statute) either to retain students that had graduated that year for their preparation to a scientific activity, or to commission them as microbiologists and even not as plant physiologists, — no serious preparing of cadres whatever could be found even here.

In other words, pre-revolutionary Russia was deficient in that which science should give to its land.

A. Main trends of Soviet soil microbiology

When, together with the October Revolution, new life began building, microbiology began rising too; agronomical micro-

¹ S. A. Severin («Viestnik Agrobact. Stanz.» 16, p. 2, 1909) writes: «If Prof. Winogradsky, being head of that section, has presented agronomics with a highly valuable gift by his doctrine of nitrification, this has to be referred to the lucky star of agronomics, which had directed the brilliant mind of Winogradsky to the study of nitrification, otherwise he could have had the right, just as successfully, to render happy some other section of knowledge, having no relation whatever to agronomics».

biology as such got also soon to be determined. Its birth had not been very smooth, for scientific agronomics could not find at once the way it had to follow.

The chemization of agriculture was, at first, undervalued by a great many eminent agronomists. It was quite usual to oppose the raising of the natural productive forces of soil to the application of artificial fertilizers. Attaching great importance, in the rise of the yielding capacity, to the heightening of genetic virtues of cultures and to their conformity to the climatic zones of the Union, a great many people undervalued the importance of agrotechnical measures for the development of plants.

The task of finding the right orientation by selecting, as the main trend, the one lying in the direction of a narrow contact with chemization was easier for those scientists, who worked in Moscow. Here C. A. Timiriazeff's word and deed were easier to be felt; here D. N. Prjanschnikov and his school were disseminating the ideas of chemical agronomy; here J. V. Samoilov had founded the Institute on Fertilizers, one of the first important Soviet scientific institutes. But no microbiological scientists could be found in Moscow at the very beginning. Everything had to be started anew. One had to learn, to create a new trend, simultaneously carrying out work and preparing new cadres. All this, naturally, hindered work.

If we succeeded in passing over, in spite of great difficulties, to a new creative work and organized it anew on a new scale, this was conditioned by the peculiar character of Soviet development and, in particular, by the system of combining theory and practice, so characteristic of socialistic construction. We not only apply in practice correlations, formerly discovered by science, and utilize our own scientific experience, as well as that foreign. Neither is our practice limited to serving current needs of individual institutions — the carrying out of all kind of analyses, and so on. Recognizing all the above enumerated to be of an extreme importance, together with disseminating and developing it, we still more deeply apply the conjunction of theory and practice. Thus, for instance, in the application of fertilizers, we utilize all the achievements of science and this helps us to make our first steps. Yet the very first steps of our work applying in something already known, give us new possibilities, we get familiar with new soil types and their changes due to the interference of science. Thus, new problems arise which, when being solved, give further light and progress to both theory and practice.

We are no more troubled by the multiformity and variety of local conditions in different parts of the Union, and we see in this, on the contrary, new possibilities for the success of our common purpose. The unbounded expanses of our Union rapidly accustom us to understand that each trite method has a limited circle of application. If we want to make theory pre-

ciser, or check it, we have no difficulty in finding some place of the Union where it is most expedient to do this.

Definite correlations between the centre and the periphery result from this. Periphery is not only a place for the application of that which is ready, but it gives new motives; it is not a mass commonplace analyst that is wanted there, but a most refined expert. If the latter has no answer to give, he scientifically formulates a new problem.

It is perfectly natural that our new born soil microbiology could not at once play a leading part in agronomics. But at any rate, the problems clearly formulated by agronomics have already been thoroughly theoretically elucidated, and the essential most important problems of the present day have approached practical solution.

As mentioned above, the problem of utilizing the root nodule bacteria had hardly moved forward in the tsarist period¹. The Agrobacteriological Laboratory in Moscow manifested of an extremely rapid theoretical and practical (see works of V. P. I s r a i l s k y) progress in that problem. It not only produced new theoretical works but also set going the production of nitragin. As far back as 1930 it produced nitragin for 250,000 ha. Later on it transmitted its production to laboratories, immediately serving the principal regions.

Endeepened work for the solution of new problems concerning this question as, for instance, the reason why the soya in a number of cases readily forms root nodules, but does not assimilate nitrogen upon the chernozem in Ukraine, has been transmitted to the Microbiological Institute of the Lenin Academy and to respective special institutions.

The other problem in which old Russia had been backward was the rationalization of stable manure and peat fertilization. Besides the Scientific Institute on Fertilizers, the Peat Institute of the People's Commissariat for Agriculture and a series of regional institutions, microbiologic research on that problem, as being one of the most essential was undertaken by the Institute of Agricultural Microbiology of the Lenin Academy, which succeeded in bringing the investigation of respective problems to an application of their solution to practical farming.

The third problem, brought by the Soviet microbiologists to the stage of introduction into practice, is a new problem particularly put forward by the specific needs of socialistic agriculture. Connected with the problem of a scientific guidance of

¹ This corresponded to the extreme backwardness of Russia in the culture of the leguminous. Such a figure as 50,000 ha. under kidney-beans is simply ridiculous. The general area under the leguminous — 3,000,000 ha. is no doubt, less than what it ought to have been, issuing from the calculation to the tilled area and correlations in world agriculture.

socialistic agriculture being in motion, connected with tempoos of the socialistic agriculture, the microbiological evaluation of the requirements of the fields in fertilizers, as well as the diagnostics of their present condition, acquire a gradually increasing importance (see below).

Each of these practical problems has an enormous significance. It should suffice to point out that our agricultural economy gives every year more than 300 millions tons of manure containing 1.5—2 million tons of nitrogen. Thus, even if taking nitrogen alone, the obtained manure corresponds to the world production of nitrogenic artificial fertilizers. Meanwhile, half of the nitrogen gets lost due to microbiological processes being non-regulated in the storing and preparing of manure and in the taking it out into the field. The root nodule bacteria give the USA, as computed by different authors, from 1 to 2 million tons of nitrogen a year¹. Why should they not give the same to us too?

Yet, these problems of root nodule bacteria and manure are but of a limited importance in our perspective plan for the future. One should not forget that even poor podzols contain nitrogen in an amount which might suffice for giving good yields during 200—300 years, the question not only being in that there lie dead stores, neither in that we want to utilize them, but in that also, that only $\frac{2}{3}$ of the fertilizers introduced get utilized, there existing at the same time microbiological processes giving gratuitous nitrogen. If possessing a number of favourable conditions we obtain an unfavourable balance, it depends on the existence of disadvantageous processes and that we have not mastered these, as a whole, it not only being necessary to know how to mobilize nitrogen, but also — how to do it in time. The phosphorus problem, in its turn, puts forward a series of microbiological problems.

These problems had been well realized by Soviet agronomics, but a scientific microbiological formulation of them was required. Thus, the problem of nitrogen mobilization, at this or other time, may be either a problem of an actual mobilization of nitrogen, either one of the struggle with demobilizing processes, or of both of them taken together.

Therefore, the way towards the managing of the dynamics of soil processes lies through microbiological diagnostics on the background of the application of fertilizers and other agrotechnical means. A thorough theoretical analysis of practical problems was required only for outlining the real ways for solving them. Now then, if we were to observe wherefrom proceed these or other theoretical lines of approach to new problems, we should

¹ Fred, E. Br., Baldwin, I. L., McCoy, E. Root nodule bacteria; etc. Madison, 1932.

arrive at the conclusion that it would be incorrect to wholly connect the development of soil microbiology with the request of practical agronomists and respective practical institutes. Theoretical forces and new lines of approach corresponding to new problems have been put forward in substance by chairs of universities and higher schools standing near to them (K o s t y t s c h e v — Leningrad, Z a l e s s k y — Kharkov, R i c h t e r — Saratov, C h o l o d n y — Kiev, C h u d i a k o w — Moscow, K e l l e r — Voronezh, U s p e n s k y — Moscow, and others).

In fact, microbiology that had stood, before the revolution, somewhere in the lobby, or quite outside the university doors, has entered the Soviet school-plans of the physico-mathematical faculty in the capacity of a specialty. The young specialty had to listen attentively to life and, in particular, to agronomics, the latter being of so great an importance for our Union.

Naturally, it began by transferring upon objects, connected in this or other way with agronomical practice, those theoretical moments, which were particularly valued by this or other chair.

But common to all was the tendency of bringing nearer to concrete reality the inheritance of the W i n o g r a d s k y — O m e l i a n s k y ' s school, as well as the lines of approach of the chair itself, the concretization of the cycle doctrine of the transformation of substances in nature following initially two directions: on the one hand, was endeavored the study of the mechanism and definite sides of most important processes, on the other — the real meaning was studied of this or other process in soils of different zones, the influence of different fertilizers, etc.

As a peculiar ramification of the second direction, work on the elaboration of the microbiological evaluation of the need in fertilizers, and the microbiological diagnostics of the current condition of soils should be taken particular note of.

Later on, connected with these latter lines of approach, a third direction began getting manifest in a higher degree, — the study of the morphological changes of organisms in connection with their functioning at this or other stage of the cycle of transformation of substances, in these or other surroundings.

Finally, it is but towards the second half of the first five-year plan that a trend began visibly to get outlined towards getting acquainted with the life-activity of certain soil microbes and learning to regulate soil biodynamics.

When classifying all works along the said directions I only wish to show with preciseness the correlations that had really existed. Personally, I am not an adherent of this system in work and, on the contrary, endeavour, from the very beginning, to unite these trends. But this is to be spoken of below.

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B. Biochemistry of microbiological processes

1. Phosphorus cycle

Relatively to the study of the mechanism of the most important individual processes, special attention was, naturally, given to the cycles of phosphorus and nitrogen.

One of the greatest achievements in that direction was K. I. Rudakov's discovery of the bacterial reduction of phosphoric acid. Up to that time we conceived the transformation of phosphorus to be a conversion of phosphoric acid from more soluble salts into those less soluble, as well as into organic complex compounds, and the reverse. But neither reduction, nor oxidation of phosphorus had been known to exist in organisms. It had been established that even the most complex phosphoric compounds in organisms, as, for instance, nucleinic acid and its compounds with proteins, still contain the oxidized phosphorus of phosphoric acid. These compounds when introduced into soil get split, and phosphoric acid ions are liberated, mobilized, so to say. These mobilized ions are again assimilated by the plant and, further on, by the animal devouring it. Insoluble phosphates of bones are again formed, as well as lecithins which cannot be detected by ion reactions, nucleinic acid and other organic compounds, but no instance of reduction was known.

Those preliminary considerations which had been known before and which prompted to investigate that process do not depreciate Rudakov's discovery, but, on the contrary, set off its significance. Thus, M. A. Egorov, when studying processes going on in manure, had long before observed losses in phosphoric acid. He had presumed that a reduction of phosphoric acid was taking place — up to volatile compounds. The Agro-Bacteriological Station in the person of S. E. Severin, had been occupied with the bacterial mobilization of phosphoric acid. A direct order received from the People's Commissariat for Agriculture and the Shatilov Experimental Station, conducted at that time by A. N. Lebediantzev, was to serve as the first impulse.

A. N. Lebediantzev, when studying the possibility of utilizing phosphorites as fertilizers on a degraded chernozem, had established processes of the mobilization of phosphoric acid to go on very successfully in summer, while in autumn there took place its secondary retrogradation. Supposing this phenomenon to be connected with biological activities, A. N. Lebediantzev entered into contact with the Agro-Bacteriological Station.

K. I. Rudakov's investigations absolutely prove the reduction of phosphoric acid to phosphorus. Besides, the formation of volatile compounds of phosphorus has been established. But it has not been elucidated as yet, whether these latter are

ethers of phosphoric acids as F. Liebert¹ presumes, or products of a deeper reduction of phosphates — to phosphorous hydrogen or some near compounds.

Liebert's objections, indicating thermochemical correlations which exclude, in his opinion, the possibility of a biological reduction of phosphates are not well grounded. Liebert proceeds in his calculations from summary quantities of the potential energy of all the molecules of organic compounds and phosphoric acid. Yet, the organism consumes its organic matter by individual radicals, and not at once, but through a whole series of stages, corresponding to the discharge of separate electrons. As is well known, if we subject to oxidation organic compounds of a different degree of oxidation by carbon atoms, oxidizing will first affect those of the atoms which are already more oxidized. Thus, ethyl alcohol gets oxidized up to aldehyde, and, further on, up to acetic acid, partly oxidized carbon being oxidized, whilst the methyl group remains unaffected².

This shows the energetic unequivalency of the oxidation of different atoms and of the degrees of their oxidation. Therefore, the utilization of average values, referred either to the whole of the molecule, or only to a carbon atom, cannot give an idea of the really existing energetic differences at different degrees of the oxidation of carbon or phosphorus atoms.

Carbon of the aldehyde function may readily reduce phosphoric acid to phosphorous, whilst the methyl group cannot do even that.

Partial oxidation of carbon compounds may, thus, be possible, the more reduced part of these will accumulate as respective residues, or may be oxidized at the expense of air oxygen giving a greater energetic effect, or at that of some other stronger oxidizer. That is to say, corresponding reactions may be possible in the capacity of those conjugated or of reactions giving products not fully oxidized.

Besides K. I. Rudakov's works in the study of the phosphorous cycle, another essential improvement has taken place during the Soviet time. Our agrochemists, under the leadership of M. K. Domontowitsch, have arrived at the conclusion that the problem of the phosphates' solubility should be transferred to another plane. Even a tricalcium phosphate gets sufficiently well dissolved. But the whole of the problem comes to the rate of dissolution, and the recovery of the solution con-

¹ Zentralbl. f. Bak. II Abt., Bd. 72, 1927.

² Thermochemistry, as well known, had long since begun to elaborate the problem of the amount of energy given by the oxidation of definite radicals, functions, homological differences, and so on. Russian thermochemists — Louguinin, Zubov, Sventoslavsky, and others have worked in particular at that problem. A. M. Berkenheim had made an attempt to rationalize and theoretically generalize these lines of approach.

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sumed by the plants. The mobilizing capacity of phosphate in the course of time, in the given concrete surrounding, is what should be of interest for an agronomist. This is just the problem that had been taken into consideration in the elaboration of our method for evaluating the requirement of soils in lime and phosphorus.

II. Nitrogen cycle

In order to explain the Rudakov-Liebert discussion we have had to make an excursion into the domain of energetics of vital processes in general. This moment acquires a still greater importance at the explanation of the nitrogen cycle.

But the corresponding formulations could not be elucidated at once, and it is but lately that the more important problems have come near to their solution. Besides, it is but one of the moving springs. The microbiologist must take into account that he has to deal with organisms. Thermodynamics is far from being sufficient for his purposes. It is not possible to understand either the behaviour of the organism, or the course of individual vital processes without the doctrine of evolution. Much may be given in that respect by comparative physiology, as well as by ecology and by research work using as a background human interference, cultivation of land, and by other similar lines of approach.

Hence, we shall keep to the same order, as in the case of phosphorus, at the exposition of the chapter on the mechanism of transformations of the nitrogen compounds, i. e., noting, by the way, the corresponding thermodynamical lines of approach, without unfolding the whole picture. This will be given in the final chapter together with the summing up of ecology, coenology and other biological lines of approach to the soil world.

In the line of the study of the mechanism of nitrogen transformations, much attention has been given, in our Union, to nitrogen fixation and to denitrification.

1. Nitrogen fixation

Before the revolution there existed only hypotheses concerning the mechanism of nitrogen-fixation, the latter being admitted as assimilation combined with a reduction through ammonia (Winogradsky) and, reversely, as an oxidation with formation of nitrates. Ammonium nitrate was admitted by Choda't to be the first product of nitrogen assimilation. These were all ways, utilized in chemistry for fixing nitrogen. There existed no biological premises whatever.

In the year 1925 S. P. Kostytshev, Ryskalchuk and Shvetzova demonstrated that ammonia was being formed during assimilation of nitrogen by *Azotobacter agile*. The same authors established later on (1926) that the Azoto-

bacter not only does not form any nitrates, but, on the contrary, energetically reduces the latter to ammoniac. The *Azotobacter* reminds, in that respect, the behaviour of moulds. Kostytschev and Tsve tk o v a having formerly (1920) elaborated for the latter methods for distinguishing ammonia, obtained by way of reduction with excess of energetic material, from that obtained by way of desamidation, — this error could be eliminated.

In 1931 Kostytschev and Sheloumova published a new extensive investigation on the same subject with the *Azotobacter vinelandii*. They had again detected marked quantities of ammonia in energetically growing cultures, in the presence of an excess of energetic material.

In 1929 A. R. M i n e n k o v repeated Kostytschev's experiments, but with the *Azotobacter chroococcum*. He could not detect ammoniac. Neither was it detected by T. T. D e m i d e n k o who used the roots of Zea Mays for his experiments.

D. M. N o v o g r u d s k y applied the blowing through and the Nessler reagent for detecting ammonia in cultures of *Az. chroococcum*. Besides, he made *B. denitrofluorescens* and *B. Stutzeri*, which exhaust ammonia to 0.001 mg. D. M. N o v o g r u d s k y succeeded in detecting ammonia by both these ways in energetically growing cultures in the presence of an excess of energetic material. Yet the amount of ammonia was insignificant — from 0.001 to 0.01 mg.

The discussion at the Soil Conference in Jan. 1930 has shown that some of our other investigators had suffered failures of the type of M i n e n k o v's failure with the *Az. chroococcum*, the question being not only in the small sensitiveness of the applied reactions for ammonia, but also in the strain of the *Az. chroococcum*. That of N o v o g r u d s k y should be referred to the acid producing strains.

Thus, the fixing of nitrogen through oxidation to nitrates has been definitely excluded, not only for the anaerobic *Clostridium Pastorianum*, but also for the aerobic *Azotobacter*. It seems that compounds with ammoniacal nitrogen may be considered as the first product of nitrogen fixation. Tests carried out till the present cannot decide whether free ammonia is really produced, i. e. whether the process takes place, so to say, without a direct contact with the protoplasm, or some other more mobile groups arise in the protein besides its well known amine, amid and other similar compounds. S. P. K o s t y t s c h e v has shown that ammonia detected under conditions of growth may be sharply distinguished from that obtained by way of desamidation. But desamidation is one thing whilst the splitting off from unstable groups of live protein is another. S. P. K o s t y t s c h e v is right to emphasize that the elucidation of the way

followed by nitrogen fixation sets in a novel fashion the problem of the economy of this process. Yet two reservations are to be made.

First of all the fact that this fixation takes place through ammonia does not prove at all that the process follows the scheme of Haber's synthesis. It is most probable that in the case of *Azotobacter* the carbon of mannitol reduces nitrogen, and after the latter has become active it attracts hydrogen. Oxygen, which cannot be dispensed with, is necessary for the consuming of tighter functions and for attracting the less mobile electrons whilst the more mobile electrons partially are fixed by nitrogen, which though attracting them less than oxygen, yet gets its share too. Kostytshev himself emphasizes (1931) in the last of his published works that in the case of nitrogen assimilation we have a process of nitrogen reduction, similar to that of the reduction of nitrates.

The same scheme may be followed in the case of the anaerobic *Clostridium Pastorianum*, but with the common to anaerobic processes distinction that less mobile electrons are not caught by oxygen but by more readily reduced carbon atoms of the same sugar.

In that case, the molecular hydrogen expelled has, in fact, nothing to do with the process, just as in the case of the reduction of sulfates has been proved experimentally. By the way, according to the newest data, the whole matter is in the nitrogen activation, in the Haber synthesis as well. But it is reached there under conditions not realizable in organisms.

Yet even if the process were to follow Haber's scheme, to the corresponding thesis of S. P. Kostytshev another reservation is to be made. In production, when working after Haber's system, certain difficulties arise at the production of molecular hydrogen, causing some expense. Hydrogen must possess a certain significance too for microorganisms. Hydrogen in mannitol is not quite the same thing as molecular hydrogen. The mannitol hydrogen is not a hydrogen ion of hydrochloric acid, yet the electron in it is already displaced. The mannitol hydrogen requires reduction in order to become molecular hydrogen. This is possible at the expense of carbon only with an extremely non-economic dispersion of electrons. Therefore, the fact itself of the liberation of molecular hydrogen by *Clostridium* manifests of its working non-economically, if we take into account the expense of nutritive material¹.

¹ Butyric acid and like products are useless for *Clostridium*. If we take into account the store of energy in these products and calculate the per cent of sugar energy that has again been converted into that chemical, we shall have to come to a reverse conclusion relatively to the thriftiness of the process.

In short, the molecular hydrogen cannot be considered to be a gratuitous product—it has to be prepared. Hence, even in the Haber scheme, one may speak of the comparative economy of the processes of nitrogen fixation, as they may differ in methods of preparing hydrogen.

If the isolation of ammonia into the outer medium is not a proof of ammonia being, as such, the first product of nitrogen fixation, it has still a very great importance, from a practical point of view, for understanding the correlations between microbes and the higher plants (see below).

Naturally it is not our attention only that has been drawn by the process of nitrogen assimilation. A great many researches have been devoted to that problem in other countries too, the essence of the mechanism of the process having been studied in different ways. Burk notes the dependence of the process on the pressure of nitrogen within the limits from 0.05 to 5 atmospheres and its direct proportionality to this pressure. Barthel has elucidated the dependence of the growth of *Bact. radicola* on the pressure of oxygen. The importance of calcium is outlined (Burk, Burgess, and oth.).

The Microbiological Section of the Second International Congress of Soil Science (1930) assigned a special sitting to that problem, the study touching also upon the other side of the process—the oxidation of organic matter. The work of the Japanese investigator Aso, who had studied products of the oxidation of mannitol, presents special interest in the latter direction. There has been established the formation of phthalic acid which is of no small consequence if we take into account the rôle of phthalimide in organic chemistry. But S. P. Kostytshev proved nitrogen to be first converted into an ammoniacal form.

Still more, the way through reduction was undoubtedly proved.

Winogradsky, it is true, had long since declared himself for the reduction of nitrogen at its fixation, but one should not forget that Winogradsky had to deal with the anaerobic *Clostridium Pastorianum*, liberating hydrogen. It is very far from this to the reduction of nitrates and of the gaseous nitrogen by the aerobic bacteria just mentioned. And the recent Winogradsky's researches appeared six years after those of Kostytshev.

2. Denitrification

Great attention has been given to denitrification by the Soviet agronomic microbiology. This will be easily understood, if we take into account the data on the spread and rôle of this process (see below). M. P. Korsakova's works are to be specially noted amidst the purely biochemical investigations of the mechanism of denitrification.

M. P. K o r s a k o v a has shown that denitrifying bacteria may be divided into two groups, one of which is not capable of fermentation. Free oxygen being absent, they can yield hydrogen to nitrates, but to no other part of organic matter. Therefore a determined dependence exists between the amount of reduced nitrates and the quantity of the oxidized carbonaceous compound.

Denitrifying bacteria capable of fermentation, nitrates being deficient, may continue reducing the energetic material. Therefore no simple dependence exists here between the reduced nitrates and the sugar consumed or some other carbonaceous compound. M. P. K o r s a k o v a has investigated in special works different cases of the reduction of nitrates by organisms capable of fermentation; she has shown, further, that denitrification produced by one and the same microbe, from nitrates to free nitrogen, does not perform this way all at one time. The process, on the contrary, is divided into stages. The stage from nitrates to nitrites has been demonstrated by M. P. K o r s a k o v a with an extreme clearness.

Thus, in a series of tests, out of 14 mg. of nitrates of the initial medium, about 10 mg. could be detected in the same medium in the form of nitrites. Figures, in the cited case, are of such an order that one has to believe them, in spite of their having been obtained for an outer medium. Besides, the accumulation of nitrites in such a quantity in an outer medium indicates another fact of the greatest importance. M. P. K o r s a k o v a does not emphasize it, evidently due to that the same may be observed with other microbes and in other processes, and we somehow undervalue this phenomenon, being used to it. But it is perfectly evident that nitrites do not hold on in the cell and, seemingly, are in some way or other expelled out of it.

If we ask whether this is obligatory or not for an intermediate product, we shall, certainly, have to give a negative answer. Besides, the reduction from nitrates to nitrites requires a great amount of energy. Further on, the process of reduction does not require such energetic tensities. Therefore, if nitrous acid remains unimpaired, this may happen only if it is expelled outside the sphere of action.

Proceeding to further phases of denitrification M. P. K o r s a k o v a established the next phase of it to take place before the whole quantity of nitrates has been converted into nitrites. Nitrites obviously begin their further transformation, and free nitrogen begins getting formed. But the increment of the quantity of nitrites primarily covers their decrease, and their quantity continues increasing. The quantity of nitrates reaches its maximum towards the moment of the complete disappearance of nitrates, after which it begins markedly falling (see Fig. 1).

M. P. K o r s a k o v a established an extremely curious fact.

The sum of the nitrogen of nitrites and of that formed from them towards this time proved to be considerably smaller than the quantity of nitrogen primarily existing in the nitrates and that quantity of it, which is liberated as free nitrogen towards the end of the experiment. This deficit of the computed nitrogen (in the state of nitrates, nitrites and free nitrogen) is manifested still earlier, earlier even than the gaseous nitrogen begins getting liberated. Yet, at the marked fall of nitrites the conversion of nitrogen into some unknown compound tells with a peculiar sharpness. What sort of compound should it be?

This problem remains open to question, up to now. Despite numerous attempts, M. P. K o r s a k o v a hasnot succeeded in

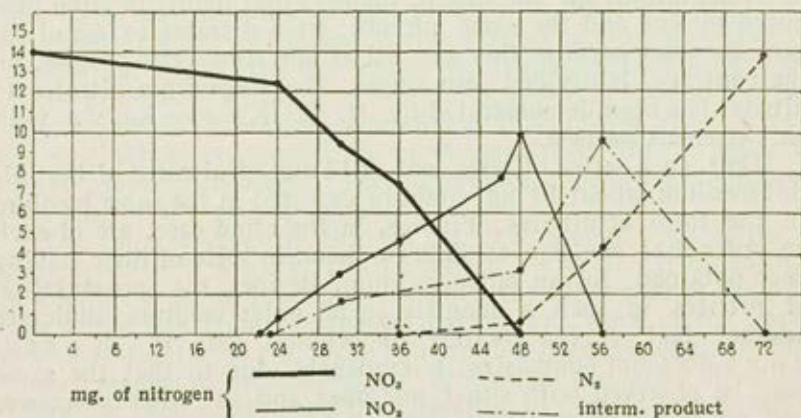


Fig. 1.

immediately proving the formation of any definite compound. B l o m, working at that problem in Denmark, has neither obtained any convincing results.

One cannot help agreeing with M. P. K o r s a k o v a that one should not tend to the extreme, trying to unify the work of denitrifying bacteria. The old group of microorganisms of denitrification has been divided, as shown above, into two groups by M. P. K o r s a k o v a: those capable of fermentation, and those incapable of it. M. P. K o r s a k o v a has established amidst the former a number of types, as for instance, those liberating hydrogen, and those not secreting it. Besides the old group of indirect denitrifiers, reducing nitrates to nitrites and liberating free nitrogen only in the presence of amides, other denitrifying bacteria are known, reducing only nitrites. Further on, M. P. K o r s a k o v a has detected organisms, which, reducing nitrites, convert them into some unknown substances and do not liberate free nitrogen at all.

Finally, there exists a group of denitrifying bacteria which oxidizes sulfur instead of organic matter. This group is also wi-

dely spread in our soils. A. G. Salimovskaya has been lately studying it in our Union. A great many other groups denitrify too under certain conditions. S. I. Kuznetsov has found the conditions under which urea bacteria denitrify too. It is beyond doubt that a still greater multiformity will be detected, if greater attention is given to the other side of denitrification, namely, to the oxidizing of organic compounds. This may be partly gathered from M. P. Korsakova's works, as well as from former Russian and foreign data. Thus, it has long since been known (van-Iterson, Naguibin) that there exist denitrificators that destroy cellulose. Besides, even closely related compounds, as for instance, calcium tartrates and calcium citrates, are affected in a different degree by different kinds of bacteria. Some prefer the first salts, others—the second.

It will be examined in a respective chapter, how is this multiformity to manifest itself at our efforts to concretize the course of the nitrogen cycle in field conditions.

Here we shall but emphasize that this multiformity gives a new clue to the understanding of the nature of the process. The fact that these differences concern such near compounds, as tartaric acid and citric acid salts, allows to utilize to a great extent the newest achievements of structural chemistry and physical chemistry in general. Such conditions make it easier to approach the problem on the basis of the doctrine of the mobile physio-chemical equilibrium as well.

3. Urea decomposition

Microorganisms capable of decomposing urea are well known to be very widely spread. The majority of higher plants contain urease-enzyme, which accelerates the transformation of urea into ammonium carbonate. Organisms are more seldom met, which do not contain urease in marked quantities and, therefore, capable of accumulating urea¹ (for instance, fungi of the type of *Lycoperdon*). Different types of bacteria relatively to urea have been studied by N. N. Ivanov (Ivanov and Smirnova, 1927).

Urea in vegetable organisms may originate in two ways: first of all it may be formed in the decomposition of proteins as a result of the splitting of arginine by arginase. This is the most widely spread and essential way. Separate stages of that process have been investigated by A. R. Kiesel. Secondly, the synthesis of urea from vapors of ammonia and from salts of ammonium is possible in higher fungi poor in urease. The

¹ Fosse, R. I. L'Urée, 1930; Kiesel, A. R. Ergebnisse d. Biologie, 1927. Ivanov, N. N. Biochem. Zeitschr. CXXXIV (1923); CXXXV (1923); CXXXVI (1923); CLXXV (1926); CLXXXI (1927); CXCI (1928); CCI (1928).

latter case has been confirmed by the successive works of N. N. I v a n o v, who elucidated, together with it, the fundamental conditions of this synthesis. For the urea synthesis oxygen is necessary, as well as a deficiency in carbo-hydrates, just as for the formation of asparagine and glutamine.

According to N. N. I v a n o v's investigations, no bacteria are capable of the urea synthesis. But there are such, which either do not contain any urease or contain only small amounts of it and may, therefore, accumulate urea obtained by the splitting of proteins. *B. megatherium*, *B. tumescens*, *Proteus Sophii*, *A. mesentericus*, *B. subtilis*, *B. mycoides* have to be referred to this group, i. e. very important soil organisms. These organisms contain enzymes, hydrolizing proteins with liberation of arginine, as well as arginase which splits off urea from arginine.

The second group — *B. fluorescens* and *B. coli commune* — are capable of splitting off and accumulating urea from arginine, yet, in decomposing proteins, they do not liberate arginine nor form urea. Finally, the third group, to which so common a microbe as *Proteus vulgaris* is to be referred, contains a full set of enzymes, urease including, allowing them to decompose proteins with further decomposition of urea formed from arginine — to ammonia.

But even bacteria, accumulating urea, such as *B. mycoides*, split it asunder, as soon as they may dispose of carbo-hydrates.

One has not, therefore, to be troubled about the urea decomposition to take its course. The breakdown of urea may be easily produced by almost any organism, at those concentrations at which urea may exist in soil under field conditions. The so-called *Urobacilli* or *Urococci* and the like are of significance in as much as we have to deal with great concentrations of urea, when, at its breakdown, the accumulation is unavoidable of great doses of ammonium carbonate and, consequently, a marked leaching.

In virtue of these correlations, urea microorganisms prove to be, in the strict sense of the word, organisms standing alkalization. These organisms may be of a special importance in agriculture, chiefly, in the keeping of stable manure, in the preparation of compost, etc., it being necessary, instead of stimulating these organisms, on the contrary, to keep them back and diminish the loss of nitrogen in the form of ammonia.

Investigations of the Soviet microbiologists have also elucidated, to a considerable degree, the physico-chemical conditions (pH and rH) under which urea bacteria work, and have established a connection of this process with denitrification (S. I. K u s n e t z o v, 1930). See below (chapter D. III). R u b e n t s c h i k (1926) has shown that urea bacteria secrete urease even in the absence of urea.

Mischustin has studied the problem of the comparative value of the sources of carbonaceous nutrition for urea bacteria.

Mischustin, tending to expand the number of index organisms, turned his attention to the group of urinary bacteria, utilizing organic acids better than carbo-hydrates. Cultures were carried out with 0.5% solutions of glucose, of cane-sugar, of maltose, of glycerol, of mannitol, of sodium tartrate, of sodium oxalate, of sodium acetate, of sodium succinate, of sodium malate, and of sodium citrate. Glucose proved to be a worse source of carbon than even oxalic acid. Acetic and citric acids had a particular nutritive value, citric acid being more active than that acetic. The work cited is not as yet brought to its end.

4. Ammonification

The study of the mechanism of the ammonification process is of an extreme interest from the standpoint of the understanding of the correlations in soil and stable manure. This problem, unfortunately, has little been studied, both in the West, and in this country.

One of the most important rules established in relation to this, is that the decomposition of proteins and ammonification are retarded by sugars and, especially, by glucose. Former suppositions about acids, formed by sugar, playing the essential rôle in that case, meet with objections. Thus, Sears¹ has shown that the addition of chalk does not eliminate the action of glucose. This objection, certainly, cannot be strict to the end. Only the outer medium is, beyond doubt, neutralized by chalk. Yet, the majority takes this objection into account, and Waksman² extends to bacteria the explanation which follows from V. S. Butkewitsch's³ data.

In his opinion, in the absence of sugar, nitrogenous material is utilized not only as such, but also in the capacity of energetic material. As a result of this, more protein has to be expended and nitrogenous residues of consumed aminoacids are liberated as ammonia.

This problem, relatively to *Bac. mycoides*, has been investigated in our Union by H. Glinka-Tschernorutzky (1929). She has shown that if, glucose being absent, 6 mg. of ammonia are formed in 8 days to 1 mg. of nitrogen of the bacteria body, glucose being added, only 0.85 mg. of ammonia will be formed under the same conditions. Glinka-Tschernorutzky has demonstrated that the retardation in the splitting of protein in the presence of sugar, cannot be explained by

¹ Sears. J. infect. Dis., XX., 103(1915).

² Waksman and Lomanidze. J. Amer., XXX, 215(1925).

³ Butkewitsch. Jahrbücher f. wiss. Botanik., XXXVIII 147—240 (1903).

retardation in the work of enzymes hydrolizing proteins. In consequence, she assents to Waksman's opinion that glucose, giving a good source of energy, makes it superfluous to decompose a great amount of protein.

As we know, the splitting off of ammonia is possible from amine and amide groups. Whilst ammonia is split off from amide groups without the disintegration of the whole molecule by enzymes of a hydrolizing type, it is liberated from amine groups only under conditions of deep oxidizing decomposition. The *Bac. mycoides*, probably, does not possess desamidase, as it liberates ammonia only at the combustion of nitrogenous material.

This might be the reason of the absence of tests, proving that *Bac. mycoides* may utilize ammonia in the capacity of a source of nitrogen. According to Glinka-Tschernorutsky's data, it assimilates badly even monoamino acids.

All the above mentioned data, on the one hand, have to be checked, the oxidation-reduction conditions being taken into account; on the other hand, it should be taken into account at the calculation of the ammonifying power of *Bac. mycoides*.

5. The Emscher processes

It is most probable, that losses of nitrogen in stable manure, etc. may be connected with processes studied on the so-called «Emscher wells» serving for the biochemical transformation of residues of sewage liquids. Bach and Sierp¹, and others have shown nitrogen, liberated in this case in a gaseous form, not to be a product of denitrification, but one resulting from amine and other groups of products of the splitting of proteins. These processes take place under conditions of high reductability, when denitrification is nearing its end, due to the absence of nitrates and nitrites. Of Soviet works in this field that of K. N. Korolkov (1926) is to be noted, as very circumstantial; in spite of its having been printed with a considerable delay, it contains a series of data substantially supplementing Bach and Sierp's works (K. N. Korolkov's work had been carried out earlier), and establishes, besides, a series of essential new data.

The following out of K. N. Korolkov's deductions is to be noted: 1) The normal fermentation of the Emscher residue presents an anaerobic decomposition of the organic matter, in which no accumulation of fatty acids takes place in the liquid surrounding it, due to their decomposition to methane, carbon acid and hydrogen. 2) Acid fermentation is also an anaerobic decomposition in which the organic matter is decomposed

¹ Bach und Sierp. Centralbl. f. Bakt. II Abt. Bd. 58 (1923); Bd. 60 (1924).

only up to fatty acids. 3) The maintenance of the alkaline reaction ($\text{pH}=7.8$) is a condition favourable to the work of microorganisms, splitting acids. This reaction is connected, in its turn, on the one hand, with the rate of the decomposition of salts of acids to gaseous products, on the other, with the buffering capacity of the media.

As we see, the first thesis of Korolkov brings the Emscher processes near to W. L. Omeliansky's¹ investigations concerning the decomposition of the formic acid and of compounds similar to it.

It is to be pitied that K. N. Korolkov's work is but of a purely chemical character, and organisms, as such, have not been investigated in it.

III. Sulfur Cycle

The sulfur cycle is going on in soil in two directions:

1. in the direction of hydrogen sulfide formation;
2. in the direction of the oxidation of hydrogen sulfide to sulfates.

The formation of hydrogen sulfide with the assistance of microorganisms, as had been established to a considerable degree by Russian authors, may be of two types: a) an anaerobic decomposition of proteinic compounds; and b) a reduction of sulfates and related compounds.

One thing only is clear: due to the complexity of transformations taking place in putrefactive decomposition, it cannot be considered as a simple splitting off without the reduction of sulfur, as not the whole of sulfur in protein is completely reduced. Thus cystine, undoubtedly, contains sulfur in a partly oxidized form.

We have no other preciser data on the chemism of the sulfur reduction in putrefaction.

Our literature is richer in data on the reduction of sulfates, but these data are little connected with soil microbiology (Rubentschik, Tausson, Issatschenko, Salimovskaya). However, we have to dwell on them, having in view that in many cases, within the province of agricultural microbiology, the reduction of sulfates must be taken into account. The reduction of sulfates is always conceivable in soil and is to be observed in the presence of a great amount of organic matter and an excess of moisture, i. e. on peaty lands, everywhere on flood-plains, as well as upon other soils, in regions of excessive moisture (for instance, the region of the Western district Station and in White Russia).

¹ See the summary of W. L. Omeliansky in «Lefer's Handbuch d. techn. Mykologie», B. V (641—653).

The following works should be noted, as putting forward new principles for the theory of the sulfur reduction.

Rubentshik (1928), has established, mainly in connection with the study of the liman curative muds, that reduction of sulfates is not connected with the formation of methane, as had supposed Hoppe-Seyler.

V. O. Tausson (1932) has not only summed up the material on the energetics of the sulfates reduction, but has also shown the possibility of reducing sulfates at the expense of the oxidizing of hydrocarbons, expressed by the conversion of the open-chain paraffins into ring compounds. This type of conversion, established by V. O. Tausson in connection with microbiological modifications of petroleum, can certainly occur upon peaty soils and in other similar cases.

No data have been published on the chemism of sulfur oxidation since 1917, as to literature on the spread of this process—see chapter C.

IV. Carbon cycle

In as much as carbon is the essential building, as well as energetic material, we had to touch upon its transformations, when speaking of other cycles. The conclusive chapter will also embrace the most important moments of the carbon cycle. Besides, should we widely approach this problem, we must include in it almost the whole of biochemical microbiology.

This chapter, therefore, will be concerned but with two items: first we shall examine those processes which are in immediate touch with soil microbiology and the study of which has been originated, to a great degree, by agronomics. Secondly, we shall refer to such works in other domains, which may advance soil microbiology, but have been little utilized as yet.

The most important compound, subjected in soil to decomposition, is cellulose. After the classical works of W. L. Omeliansky (1902), who had studied the anaerobic decomposition of cellulose in the absence of nitrates, little study was devoted in our country to the stages of cellulose decomposition and to the purely microbiological side of the matter. It is chiefly in the West that the aerobic decomposition of cellulose¹ and that anaerobic, in the presence of nitrates, have been established and studied.

We have in this respect the data on the spread of the processes of the cellulose breakdown examined in chapter C.

Out of works, establishing the essential moments of the chemism of the decomposition, one should note the work of B.L.

¹ See: Waksman. Principles of Soil Microbiology (1932).

I s s a t s c h e n k o (1921) who was the first to establish sugar, as an intermediate product in cultures of the cellulose⁶ decomposing aerobic bacteria.

Transformations of the soil organic matter, in the proper sense of the word, have also been much more studied in the West than in this country, the prevailing trend being purely chemical. Thus was grounded the significance of the ratio C:N for the losses and accumulation of nitrogen¹. Besides American authors have given special attention to, so to say, the organic chemistry of the soil and to the setting free of individual chemical compounds in soil in different processes. A. A. S h m u k ' s works should be noted among the Soviet purely chemical works on soil organic matter.

A special trend has been created by the study of microbiological transformations of organic compounds difficult to oxidize. This trend, primarily arisen in the West², has progressed in the USSR with particular success. It comes into contact with problems of soil microbiology along two lines.

Firstly, no few compounds are met in the remnants of life, getting into soil, such as wax, fatty acids, aromatic compounds and oth., the microbiological decomposition of which is, doubtless, going on.

Secondly, antiseptics — phenols and a whole series of compounds of a similar character — have been applied for partial soil sterilization; they have been noted to disappear in soil, the rate of this disappearing increasing with each repetition of the experiment.

Researches in that direction have been started in our Union at the Moscow State University. When conducting pot culture experiments in paraffin and paraffined vessels in a greenhouse I succeeded in isolating *Aspergillus flavus* and some other organisms, which hindered very much my work by rapidly disintegrating paraffin. In 1923, V. O. T a u s s o n began working at my cultures and, later on, successfully developed these researches as collaborator of our laboratory of the Timiriazeff Biological Institute. V. O. T a u s s o n isolated from different soils, chiefly, in rock-oil regions, a series of organisms, oxidizing not only paraffins with an open chain, but also a great many ring compounds. He described in detail a whole series of bacteria, oxidizing naphthalene, phenanthrene, benzene and related compounds. There are indications, in T a u s s o n ' s latest work, of microorganisms, oxidizing polymethylene compounds and different hydrocarbons with condensed benzene ring nuclei (diphenyl, dibenzyl, stilben, anthracene).

¹ See: Springer, O. Bodenkundliche Forschungen, III, 39—70 (1932).

² R a h n. Zentralbl. f. Bakt. Abt. II (1906). See also: W a k s m a n ' s Principles.

One of the stimuli for working in that direction was the tendency to study microbiological processes for such type of compounds that had been relatively little studied, in order to obtain in that way, a fuller conception of these substantial and energetic transformations, of which carbon compounds are capable.

We know, thus, for instance that, under conditions of life processes there continually take place transformations of open-chain compounds into ring compounds, and vice versa. Yet we do not know, in the majority of cases, the courses that these processes take. The benzene ring in particular had been so little investigated up to the present time, that a great many people took ring compounds for final products of life activity, for waste, and even organisms accumulating these compounds were considered by some to have finished their evolutionary course. Though sufficient data, contradicting this thesis, had been known long ago, and Nägeli had already described a series of cases of the splitting of ring compounds by organism, it is but at the present time that a categorical turn has taken place towards the acknowledgment of a great mobility of ring compounds in organisms and towards the study of different means of the transformations of ring compounds. The study of their molecule, such as it is under conditions of life processes, acquires an exceptional importance. Thus, V. S. Butkevitch has shown, that the capacity of a microorganism to split quinine acid may be utilized as a reagent for a definite type of sugar fermentation.

The oxidation of open-chain paraffins is also of a quite exceptional theoretical significance. Organisms constantly consume the methyl group, as in ethyl alcohol, for instance, in lactic acid and oth., but this process takes place on the background of others; and we usually do not catch its specific peculiarities. Yet, an analysis alone of the energetic correlations shows that particular correlations are obtained in case of accumulation of groups reduced up to the limit.

So it is that, whilst we have to deal, in the case of sugar, with a compound capable of oxidation with the assistance of air oxygen, as well as of intramolecular regroupings, both these processes giving issue to energy, — in case of paraffins, for instance, energy is set free at entire oxidation in a much greater amount than by sugar, but intramolecular processes cannot give any energy. Due to this, these substances may participate only in processes much more depending on the conditions of oxidation. These processes, therefore, as I had established it during the isolation of the *Aspergillus flavus* culture, and as V. O. Tausson later systematically proved, must have a pronounced tendency towards aerobiosis, as well as towards alkaline media.

Having studied a whole series of new groupings of carbon atoms, V. O. Tausson has proceeded now to two extremely important problems:

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1. to the mastering of the transformations of the soil organic matters and minerals, and

2. to the elucidation of the energetic possibilities which carbon atoms, of a different degree of oxidation, give to organisms.

Details may be found in the works of V. O. T a u s s o n. Here I will but outline the following moments. Oxidation does not always take the course that seems most probable on the basis of data of organic chemistry. Thus, the naphthalene oxidation does not take place either through phthalic acid, or through naphthols.

It proved necessary, for studying the enumerated processes, not only to elucidate the general conditions of these processes (pH and rH, and the like), but also to conduct experiments under quite novel conditions. Thus, for the study of benzene bacteria, V. O. T a u s s o n elaborated a special method of a «diffusional influx», solving the problem of tests with liquid hydrocarbons.

Concerning the summary on the splitting of fat, see G. S e l i b e r (1932), likewise G. S e l i b e r «Formation and decomposition of fats by microorganisms», «Monographies of the Leshaft Institute», 1926, Leningrad.

C. Spread of microorganisms

1. Origin of investigations and conditions which contributed to their development

As had been noted when reviewing the main trends of the Soviet soil microbiology (chapter A), the concretization of our knowledge on organic cycles of substances was taking place not only in the direction of the endeeptening of a biochemical conception of processes, but also in that of the study of problems, as to where this or that process actually takes place, in what soil, at what depth it plays an essential rôle, where and how it might be put under our control for the purpose of obtaining the highest economic effect. We may boldly say that 99% of all we know concerning the enumerated problems on soils of the Soviet Union has been obtained after the revolution. It is difficult to imagine now the way we approached these problems in the years of «orientation work» on soil microbiology (1918—1923), when life itself called forth work, yet no one knew how to begin it.

Towards that time the post-war revival of the interest in agriculture and in a peaceful utilization of chemization, forwarded in the West too the same problems. Works appear there of an enormous importance: C o n n ¹ (1918, America), W i n o g r a d s k y ² (1924, 1925, 1926, France), which allow, for the first time, if it may be so expressed, to «look into the soil».

¹ New York Agr. Exp. Stat. Techn. Bull., No 64 (1918).

² Compt. rend. d. Ac. d. Sc. Paris. T. 179 (1924); Ann. de l'Inst. Pasteur, 1925; 1926.

These works, making it possible to make preparations and to see microbes directly isolated from soil, have been taken up with avidity and great success in our country¹. The fact is that, in spite of all the difficulties which our native land had to experience in those years in connection with different interventions, we strived to solve the problems confronting us and came to utilize the old attempts of making preparations from soil, improving, at the same time, methods for isolating cultures of microbes from it. In particular, we had been ahead of Conn's and Winogradsky's lines of approach to problems in the understanding of the medium conditions, which allowed us to improve these methods.

The circumstances favourable to the development in the USSR of the study of the distribution of microorganisms, connected with their rôle were such:

1. We have at our disposal $\frac{1}{4}$ of the terrestrial dry land. Soviet microbiologists have investigated soils from the arctic (in latitude of 73°16', North), Nova Zemlia (Kazansky 1928 and 1932), across all zones, up to the foot of Mount Ararat and the Batum subtropics: Richter — 1925; Shulgina — 1926 and 1927; Germanov — 1927, 1928, 1932; Razumov — 1928 and 1929, and many others. The material most complete is to be found in the work of A. N. Naumova, which is now being printed in the Transactions of the Institute of Fertilizers, № 108 (1933). Turkestan (Cononova, Karpinskaya, Sheloumova, 1931; Rokitzkaya, Sabinin, Pereskokov) and the foot hills of the Tian Shan mountains (see summary of E. E. Uspensky in the «Proceedings of the Second Intern. Cong. of Soil Science»), Siberia (Davidov, 1930; Sushkina, 1932), the Far East (Khakhina, 1931), the Ural region (Sabinin and Henkel, Henkel and Zacharova) have been subject to observations, whilst the essential cultural regions of the Union have been studied along different directions by several schools.

We had to deal with different soils, under different physico-geographical conditions, with various cultural habits and different problems in socialistic construction.

2. It is most essential that these soils had been systematically studied by Russian soilscientists, they were the cradle of soil science. Besides works, the immediate purpose of which was to establish the details of the connection between the soil profile and the microbiological life (for inst. Razumov and Remizov, Sabinin and Minina, and oth.), the majority of our works have been conducted on definite soil differences

¹ The first work of that trend was published in Saratov by A. A. Richter.

taking into account those horizons from which tests had been taken.

3. I cannot say that we have always worked under conditions of a highly cultural farming. But we carried out our investigations neither on invitation of separate casual owners nor after a plan dear solely to ourselves. Our work greatly differed from the first system by that whole regions or districts and, finally almost the whole of the Union (for central institutions) were open to us, and not casually snatched out lucky plots.

Our work profitably differed from the second system, by that microbiological researches could be conducted in close contact with those adjacent. Our work runs according to a general plan of an intensive raising of the chemization of our country.

Our investigations may rest on agrochemical and on field tests, not only on standard soil researches. We are in direct connection with factories preparing artificial fertilizers, and with their scientific leaders. Thus, our Dolgoprudny Experimental Field is that of the People's Commissariat for Heavy Industry,—yet works have been conducted here also by such soilscientists as prof. W. W. Gemmerling, the member of Acad. K. K. Gedroiz, by the agrochemical school of the member of Acad. D. N. Prjanischnikow, and oth.

4. The multiformity of the conditions in our Union has set difficult problems to be solved by many methods. They had to be studied considerably deeper for giving mobile systems of definitions, adapted to modifications according to soil, region, aim, etc. Improved methods offered, in turn, new possibilities. Our Union, for instance, was the first to point out difficulties arising for Winogradsky's method, in connection with the so-called «adsorption» of microbes (N. N. Chudjakov together with Dianova and Voroshilova), but we also were the first to find an issue out of these difficulties (T. N. Germanov) founded on K. K. Gedroiz's works on the action of ions on the dispersion of soil colloids.

II. Importance of investigations

Is it possible for us to say now that a new stage has been created in the study of the soil microbiology? Does the present situation differ in principle from that which had been summed up with so much talent by W. L. Omeliansky in his manual? Certainly, we have to give a positive answer. And the possibility of directly studying the soil microorganisms by way of the development of Conn-Winogradsky's methods has become one of the corner-stones of the new edifice.

What is, indeed, the significance of new figures, obtained by new methods? There, where we had 1 million we put 2—2.5 milliards, according to Conn-Winogradsky. It seemed

to some of us that we should have to set one or two scores of milliards as the average population of bacteria per 1 g. of soil, but this did not prove to be true, when taking into account only the usual forms of bacteria. Germanov's method (see below) allowing to remove the adsorption of bacteria and to reckon the visible forms, gives usually in our soils 2.3—2.5 milliards, seldom 5—6 milliards, per 1 g. of soil. It is but in stable manure that we find 20—40—60 milliards (E. N. Volkonskaya, Scientific Institute on Fertilizers, work now being printed).

Even moderate figures, about 2.5 milliards of bacteria per 1 g. of soil, are of a great significance. This means much more than an ordinary arithmetical increase by 1000—2000 times as much as compared to the pre-war figures. The figures obtained now are so high, that even the greatest sceptics have to speak of the dynamics of soil processes, as of biodynamics.

It must be acknowledged, that we consider S. P. Kostytshev's calculations (Kostytshev and Shulgina, 1928) to be somewhat exaggerated. Microbes well fed in cultures, cannot be compared without reservation with those in soil, where they have to limit their development due to certain shortages. Therefore I am far from considering almost all of the soil phosphorus to be that of living bodies of organisms. One comes to the same conclusion when comparing figures for podzols and chernozems (E. E. Uspensky «Proceedings of the II Intern. Congress of Soil Science»). As established by Germanov and the works of the Institute on Fertilizers (especially A. N. Naumova's Transac. Inst. Fertil. № 108, 1933), we usually have for chernozems about 3—3.5 milliards of bacteria per 1 g. of soil. This does not much differ from podzols, which are considerably poorer in organic matter and in nitrogen and phosphorus.

III. Microphenology and diagnostics

But even these more moderate figures do not modify the significance of biodynamics in the dynamics of soil processes. Soil microbes do not always master all the sources of life. Yet such periods occur too. Besides, microbes are constantly retarded in their development by a deficiency in this or other substance, and their increase in number manifests, at any rate, of their finding more food at the period. We have the right to speak of microphenology, when observing the inflexions of the curve. Thus, in certain years, (for inst. 1932) the podzols of our Dolgoprudny Experimental Station begin in the spring to behave as good chernozems, but slightly reacting on nitrogen. Sooner or later, however, a deficiency in nitrogen begins to get manifest. Usually the inflexion of the microbes curve may be observed about 10 days before cereals have begun to react. The most curious is, in such cases, that it is difficult to discriminate according only

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to chemical determinations. Thus, if we calculate nitrates, we take chemically into account but the difference between the formation and adsorption at the given time, whilst the development of organisms (crops included) depends on the amount formed per unit of time. And it is just this activity in the course of time which we may account for microbiologically when making microphenological observations.

Thus, at the comparison of the course of development of denitrifying bacteria upon podzolized soils of the Dolgoprudny Experimental Station (Zacharova) with those upon the chernozem of the Shatilov Exp. Station (Potapov), we see an essential difference in their dependence on chemical ingre-

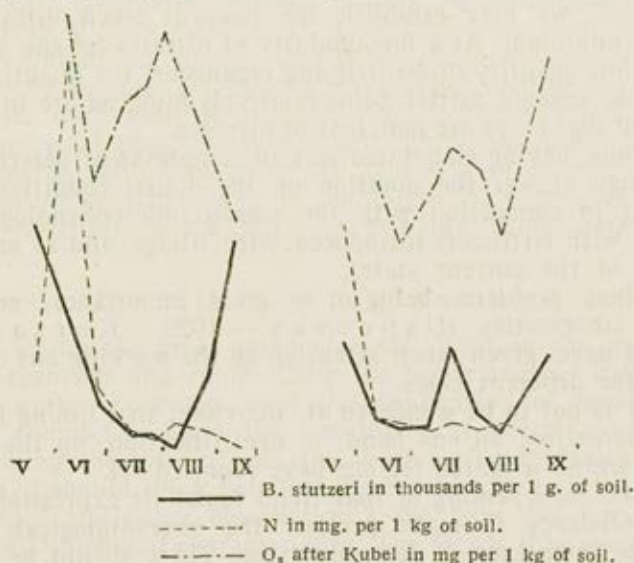


Fig. 2.

dients. In the first case, for the development of denitrifying bacteria, nitrates are to be found in the minimum in most cases, and the curve of denitrifying bacteria nearly always follows that of the nitrates. If at the housing of oats the denitrifying bacteria curve rises, the quantity of nitrates does not increase, it means just only that denitrifying bacteria consume the nitrates. In fact, at that moment the quantity of the watersoluble organic matter begins to decrease, i. e. the quantity of the other necessary component of denitrification (See Fig. 2). Yet, organic matter continued accumulating, so long as oats were snatching nitrates from denitrifying bacteria. At the Shatilov Exp. Station especially on fallow, the watersoluble organic matter is to be found in the minimum. Therefore, nitrates accumulate. The curve of the development of denitrifying organisms follows the quantity of the watersoluble organic matter.

Since spring, as mentioned above, podzols may contain a sufficient quantity of nitrates. At a certain moment, denitrifying bacteria being greatly developed, there begins a fall in nitrates, as well as in the watersoluble organic matter. At that moment we have the right to ascribe the decrease of nitrates to denitrificators. But the moment arrives when oats have diminished the quantity of nitrates so much, that denitrifying bacteria cannot oxidize the organic matter any more. The latter begins increasing, whilst nitrates decrease still more independently of denitrification.

In virtue of this, if we observe which is the curve that denitrifying bacteria follow, that of the organic matter, or that of nitrates, we may establish the moment when nitrates reach their minimum. At a low quantity of nitrates for the given soil, at a low quantity of denitrifying organisms, the quantity of the soluble organic matter being relatively high, we are in the right to say that crops are deficient in nitrogen.

Thus, having elaborated certain complexes of determinations, we may answer the question on the actual condition of soil, either in connection with the season (microphenology), or in that with fertilizers introduced, with tillage, and so on (diagnostics of the current state).

These problems being of so great importance, our principal laboratories (Uspensky — 1929, Korsakova — 1930) have given much attention to the working out of complexes for different cases.

It is not to be wondered at, therefore, that liming has attracted attention, on one hand, to denitrification, on the other, to pH, whilst irrigated regions have required rH.

The endeeptening of that trend found its expression lately in the tendency to make preciser the microbiological moments of the complexes of determinations. There should be noted in that respect, on the one hand, the clue to denitrifying bacteria by Burgwitz (1930), and, on the other, the attention to different forms of soil microbes and their cycle of development, the most conspicuous place being everywhere allotted, apart from the *Azotobacter*¹ — to *B. mycoides* and related organisms, as well as to cellulose decomposing bacteria (especially by B. L. Issatschenko and A. I. Rokitzkaya). Evidently the forms themselves in which the microorganisms get isolated, are very significative for the soil. Besides the forms of microbes, saltations (mutations) etc., the stability and variability of which is but now being studied, one may, undoubtedly utilize phases of individual development and variation of microbes, which should be studied not only in a hanging drop, in a surrounding distant from soil (see Krassilnikov, Schmalhaus-

¹ See also ch. IV.

seen), but combining observations in culture with those in soil. New bacterioscopes should be of use in that case.

A whole series of methods, the bacterioscopes of preparations from soil being of the number, have been applied up to now chiefly for the study of different soils (see below). But just these researches make it often necessary to study not so much types of soils, as the actual condition of their microflora (see below) and its dependence on tillage and fertilizers.

It is beyond doubt, that an extensive application of different methods, those bacterioscopic included (see below on methods), has already given much new material, which is now being printed (for inst., works of the Peat Institute, new works of the Scientific Inst. on Fertilizers, and others). In particular, works of the type of Conn¹ with *Bact. globiforme* have been carried out in our Union. We suppose, that it would be of a special interest to study hemp-fields in this way. When sifting soil into Petri plates with gelatine or agar, and comparing them with plates from good hemp-fields and new lands, we might notice saprophytes, which characterize the maturity of a hemp-field. By making use of such a microbe one might see what gives this or other fertilizer in the sense of bringing hemp nearer to maturity.

IV. Peculiarities of regions and soils

When seeing the great seasonal vacillations in the number of microorganisms and emphasizing the importance of that moment for works connected with the application of fertilizers, must we completely deny the possibility of microbiologically characterizing the type of soil, the physico-geographical region, and so on? I should think not. But if we were to act on the basis of prolonged stationary observations? We have too few data, as yet, for excursions characteristics. The fact is that even good indexes like *Azotobacter* may not be detected at once in a new place, which very often requires some new supplementary method. We have come to a level which usually allows of discriminating during the course of a season (see VII, 3 below), but cannot be done as yet with the simplified excursions tests.

Fortunately, observations of a rather campaign character are not required there, where are set serious economical problems. We may come to the following conclusion, when comparing observations made in different regions of our Union.

It catches one's eye, that in Northern and moister regions life is pressed to the surface (Razumov and Remizov, Sabinin and Minina). Even denitrifying bacteria are here squeezed to it (Potapov, 1930), being found in their greatest numbers at the depth of 10—15 cm. and in much lesser

¹ New York Agr. Exp. Stat. Techn. Bull. No. 172 (1930).

ones in horizon A_2 than in hor. A_1 . Life penetrates deeper in dry regions, with alkaline light soils. Microbes are detected even at the depth of 17 m. (3 000 000 per 1 g.) in the loesslike rocks of the Ural region (Winogradova, 1930; likewise Sokolov, 1932). In Turkestan we find the *Azotobacter* at the depth of 13 m. (Sabinin and Minina).

The maximum of the *Azotobacter* in such a case is usually at the depth of several meters. As a rule for the alkali soils (solontzi) in the Ural steppes the maximum of the *Azotobacter* is to be found in horizon B_1 and not in A_2 (Sabinin and Henkel, Henkel and Zacharova).

Soil, subsoil and the parent rock are difficult to be delimited in such cases.

The cold winters of the arctic (Kazansky, 1932—Nova Zemlia) do not exclude the development of a noticeable microflora in summer (up to 896 000 000 microorganisms per 1 g.¹). Yet, life towards South is definitely more energetic. One cannot think of life as advancing too slowly in regions of Turkestan (Kostytshev and Kholkin, 1930; Cononova and Sixtel; Cononova 1930, Kusnetsov²), in Transcaucasia, in the fields of the Gandzha Exp. Station (Kusnetsov, Rosenberg), as well as in the irrigated regions of the Volga Region (G. S. Zacharova, being printed in the «Transact. of the Inst. on Fert.; N. N. Sushkina, Soil Lab. of the Academy of Sciences); ammonification and nitrification take their course so rapidly that it might be necessary to think of retarding them.

It is most important that in alkaline southern soils, even after watering, a very high rH is to be observed (31 — according to S. I. Kusnetsov's observation). The behaviour of aerobes is accordingly easy to understand.

The total number of microbes in preparations in 1 g. of soil, rises up to 6 000 000 000 and higher, according to the data of Zacharova for the Valuiy Experimental Station (the Volga irrigatory region). There not being much organic matter in the corresponding chestnut soils, we actually witness cases, when almost all of it gets transformed into a living substance. And what do they mean, these 300 000 000 cells of *Azotobacter* in 1 g. of soil, even up to 900 000 000 cells of it (Naumova, Turkestan)? The *Azotobacter* is exclusively well represented in the alkali soils and the salines (solontchaks) (Keller, Germanov, Sabinin, Henkel). Quite exceptional possibilities are open to us when, in a warm dry climate, there fall on the diurnal surface powerful precipitations containing 0.1—0.3%

¹ Determinations were carried out without disintegrating the microstructures. Th. N. Germanov's method would probably give figures of twice that amount.

² See this volume p. 92.

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of nitrogen. Nitrates are then concentrated near to the surface. They may be collected, after which they again accumulate 3 times in the course of one year.

Extremely interesting data have been obtained by new methods, relatively to peat. It so happened that, just before the revolution, data on peats were summed up on the basis of old lines of approach. It was established that no microbic life existed in them, and that substances get transformed by themselves. But we are now in possession of *Begak's* data. They prove that, depending on circumstances, a layer half a meter thick is imbedded at the depth of 10—80 cm., especially rich in microbes (700 000 000—1 200 000 000 per 1 g. of moist peat). Still deeper life markedly decreases, there still remaining microbes numbering 40 000 000—1 500 000 000 per 1 g. of peat at the depth of 150—200 cm.

The *Azotobacter* (*Kharitonova*) is well developed in peat soils if they are meliorated; there is no small amount of it in preparations (about 70 000 000 per 1 g. of soil, at 20—40 million in the surrounding podzols) and pictures of fission are usual. The method of *Cholodny* (see below) applied to peats manifests of a mass development of fungi hyphae.

The same is characteristic, according to *Cholodny's* (1930) data, of forest soils, yet not being usually detected in good cultivated soils. Thus are confirmed *Waksman's* observations, characterizing uncultivated soils by an abundant development of fungi.

Winogradsky's suggestion of the autochthone microflora not containing bacilli has not been confirmed by observations in most various soils. This has been established by works of Institutes, that have carried out the prevailing mass of usual soil analyses — (Institute for Agricultural Microbiology and the Scientific Institute on Fertilizers), as well as by works on tracts of land where any contamination is difficult to be imagined. For instance, *Kazansky* assigns to the Nova Zemlia 256 000 000 bacilli out of 896 000 000 of the total number of bacteria, or 420 000 000 bacilli out of 600 000 000 of the total number.

The sharpest vacillations during the season have been observed to take place in the number of rodshaped forms, according to *Naumova's* data. This certainly makes them to be of a still greater interest. When not finding any bacilli we do not suppose them to have entirely disappeared, as in other similar cases the conversion of bacilli in other forms, is most probable (*O. T. Shulgina*, likewise other works of the Institute for Agricult. Microbiology).

V. Forms of bacteria and the non-active *Azotobacter*

Seemingly, one cannot be regardless of that a breaking of microbes into finer forms takes place in a series of cases. This,

probably, may explain cases unwillingly published, when in elective media, dilution being applied (after Hiltner), a greater number of microbes have been isolated than the total number of microorganisms visible in preparations. Or, when some specific group (of nitrogen fixing bacteria, for inst.) is sometimes taken into account, and sometimes not. In short, observations on microorganisms in different soils, as well as those on the application of fertilizers (see above) set most decidedly the problem to be resolved of the bacterial forms in soils and of the possibility of utilizing them for obtaining a better characteristics of soil.

This problem is most pronounced in respect to the Azotobacter. Cases are widely spread (Novogrudsky's summary, 1931), when we see in preparations a great many cells which are not to be distinguished from the Azotobacter, so long as the latter has not been isolated upon silica gel. In such cases, in media, containing nitrogen, an Azotobacterlike microbe can be isolated, yet not fixing nitrogen (Dianowa and Woroschilowa). Thus, the problem arose of the non-active Azotobacter and that of the Azotobacterlike organisms. It is hardly possible to solve that problem uniformly for all cases. As clearly seen in works of Novogrudsky and Naumova, an experimental error must have taken place in a number of cases, in the most typical of them. Seemingly, my indication (E. E. Uspensky «Proceedings of the II International Congress of Soil Science») that the elective media are far from being always optimal, should be taken into consideration, that soil possessing in this respect an essential importance, from which we isolate the microbes and which may give, for instance, a different quantity of iron.

At the application, in such a case, of the method of soil plates with drainage (after Uspensky and Kriutchkova) Novogrudsky and Naumova could obtain a good Azotobacter there, where it had not been detected before. The same has been shown by the new tests of our laboratory (Uspensky, 1932), as well as by works of Kriutchkova and Orentian, printed in a recent issue of the Transactions of the Ins. on Fert. (No 108, 1933). It has proved that the addition to soil of agar-agar and of silica gel secure the growth of the Azotobacter on soil plates in cases when it does not grow in spite of that a mass of it had been introduced into soil, and Ca, and P and K had been also added.

Yet, such combinations may hardly explain all cases of the non-activity of the Azotobacter. We have already noted («Proceedings of the II Intern. Congress of Soil Science», as well as the «Transactions of the Inst. on Fertilizers») the necessity of taking into account purely biological moments in that problem. The fact that a simple dilution is often of great assistance points out the rôle of the antagonists of the Azotobacter. Finally, be it under the influence of antagonists, or of some other causes, the Azotobacter

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gives seemingly invisible forms, the transformation of which into an ordinary active *Azotobacter* is not always so very simple. In short, Dianova and Woroschilowa's *Azotobacter*-like organisms exist beyond doubt. But, on the one hand, the ordinary *Azotobacter* often exists simultaneously with them—on the other hand, *Azotobacter*-like forms may be those of the *Azotobacter*.

The problem of the forms and of slow modifications undoubtedly concerns other soil microbes too. Thus, the presence of the S and R forms (Israïlsky), as well as the rôle of the bacteriophage¹ (Israïlsky) have been unconditionally established relatively to nodule bacteria. Special attention has been given to forms of the *Bac. mycoides* and of various denitrifying bacteria in connection with the study of soils and the action of fertilizers.

VI. Microorganisms and higher plants

The problem of the connection between soil organisms and higher plants has also been reflected in our literature.

A whole series of researches has been carried through in connection with the introduction of the culture of leguminous plants into our practice. Conditions have been studied of infection, of the formation of nodules, as well as the virulence and activity of these or other strains upon different soils, under definite climatic conditions of this or other region, with the addition of organic matter as well (Israïlsky, Korsakova, Lopatina, Menkina, Nitchiporovitch, Pistzov, Sabinin, Stepanova, Taranovskaya, Tchuvayev).

Further on, after the publication of Bezssonoff's² work, serious attention has been paid to the connection of free living nitrogen-fixing bacteria—to higher plants. S. P. Kostytschev together with A. Sheloumova and Protodiakonov, also Makrinov, as well as Cononova, Karpinskaya and others, carried on Bezssonoff's tests, not only with *Zea Mays*, but also with tobacco and some other plants. There seems to exist a considerable difference relatively to various higher plants. Thus, tobacco is inclined to symbiosis with the *Azotobacter*, but mustard is far from being suitable for that. Failures of Demidenko (1928), as well as of some experiments of Bezssonoff himself may be explained, on the one hand—by the strain of the *Azotobacter* and the like, on the other, by the necessity of establishing

¹ First observations on the bacteriophage in connection to root nodule bacteria are to be found in Gerretsen's, Gryn's, Sack's, Sohngens work. Zentralblatt f. Bakt. II. Abt., 1923.

² C. R. Soc. Biol. XCI, 1024 (1924); Science du Sol, VI (1927).

a mutual feeding up from the very beginning, before the utilization by the plant of the stores of nitrogen in seeds begins.

Thus, if a plant, not being sufficiently sunlit, does not supply from the very beginning an excess of sugar to the *Azotobacter*, the latter will not supply it with nitrogen by the time when the plant has utilized the supply stored in the seed.

Interesting cases of the rôle of the rizosphere have been established by A. A. Schmuk. He observed a marked reduction of nitrates to nitrites under the influence of roots. It was found that this was produced by denitrifying bacteria by their utilizing the root's secretions. Such a denitrification does not necessarily lead to losses in nitrogen and cannot injure the culture. (See also Rokitzkaya).

VII. Methods and the problems they solve

1. Study of microorganisms in microscopical preparations

The Conn-Winogradsky's method of preparing microscopical preparations is undoubtedly one making an epoch. But it required a thorough re-examination.

A whole series of authors, and especially Chudiakow's school (Chudiakow — 1926, Dianowa and Woroschilowa — 1925, Minenkov — 1928), have proved, proceeding from theoretical considerations and immediate observations that, when acting after Winogradsky's method, we do not wash off all bacteria from soil, due to their being partially «adsorbed» by it. We do not consider it correct to use unrestrictedly, in that case, the term «adsorption», as it does not give proof of anything by itself, whilst the phenomenon is not equivalent to adsorption in the physico-chemical sense of the word, and requires to be specially studied. Yet, the fact that, at the washing off after Winogradsky, the quantity of microbes detained by soil is 2—5 times greater than that washed off, is beyond doubt. If that «adsorption» is not always to be observed (Gurfeyn, 1927, 1928) and is, generally speaking, in close connection with the type of soil, it does not eliminate the problem and makes it even more difficult, as we cannot obtain any constant coefficient. Besides, it is very difficult to count up microbes when the zoogloea of the *Azotobacter* or of some other microbe is being isolated from soil. Therefore, T. N. Germanov's method of research is to be considered as an essential improvement of these methodics (1927, 1928, 1930).

On the basis of the works of K. K. Gedroiz and of our other investigators, T. N. Germanov, first of all washed the soil with a 2-n solution of NaCl for removing calcium from the absorbing complex. After this, soil is repeatedly (up to 10 times) stirred with a 0.0004-n solution of the NaOH. Thus, the micro-

structure gets disintegrated, and the organic matter is washed off from larger mineral particles.

T. N. Germanov has shown no increase of microbes to have been observed in his experiments, in spite of a prolonged process of the washing of soil with NaCl. In some cases this may, certainly, arouse doubts; yet this difficulty may be easily removed by antiseptics.

The fact that T. N. Germanov's method is, in a certain respect, a definite «extrema» is, undoubtedly, of a much greater importance. It reflects in a still lesser degree, than the original Winogradsky's method, the physiognomical features of natural soil and of the distribution of microbes, which is the reason of our preferring, for certain purposes, Winogradsky's original mode of investigation. The method of N. G. Choldny (1930) described below, is of a still greater availability for studying just the natural distribution of microbes. According to Germanov, some of the microbes suffer especially at their washing off from soil. Thus, *Clostridia* liberate spores in most cases, and nothing but scraps remain from the hyphae of fungi. But the majority of microbes preserve their life capacity and admit of germination according to A. S. Razumov's method, described below.

Apart from Germanov's method, a whole series of authors (Shulgina, 1926—1927, Razumov, 1926) introduced into Winogradsky's method different appropriate modifications into the very modes of calculation (decrease of fractions, augmentation of the quantity of initial soil, etc.) I shall not stop at these modifications, their significance varying from case to case (Henkel and Zacharova, 1930), and evidently, there may be a great many original modifications. I shall but mention S. A. Korolev's method, which removes the necessity of an exact calculation of the measured soil suspension and of the equability of the thickness of the preparation. S. A. Korolev adds to the studied soil suspension a determined quantity of the computed culture of yeast and dilutes it all to a definite volume.

After this, it is necessary to determine in the preparation the ratio of the number of computed microbes to that of the amount of yeast. Knowing the titre of yeast and multiplying it by the number found, we obtain the quantity of the computed microbes.

It proved desirable, in many cases, to check in how far microbes were alive and were they really those for which we had taken them. A. S. Razumov (1928) suggested, in such cases, to raise microbes in preparations of soil. For that purpose, instead of fixing the preparation, it is coated with agar or some other medium of elective composition. One may, thus, easily succeed in raising the *Azotobacter* and many other microbes which are then easily discriminated.

R o s s i ¹ suggested to make preparations by pressing to a clean slide fresh sections of soil, for the purpose of examining the distribution of microbes in soil and for elucidating their relation to other microbes and to separate particles of the substrate. N. G. C h o l o d n y developed and endeepened this line of approach. He digs into soil a cover-glass or an object-glass in a vertical position and, in about two weeks time, carefully takes it out with microbes sticking to it, and fixes it. This method variously modified (gluing to the glass of either fibres of cellulose or other objects) has, undoubtedly, great prospects. It is suitable not only for a the study of types of soil under natural conditions, not only for preciser observation of their actual state, but also for tests on nitrification, after W a k s m a n, as well as for other soil cultures.

H. J. C o n n in America actually begins to widely utilize this method.

R a z u m o v 's method for making preparations germinate may be combined with that of C h o l o d n y. We have already begun broadly applying it which is of great assistance in the analysis of the real relationship between microbes in soil and those in their identification, it not being necessary to have the glass dug into soil for two weeks.

Next there should be mentioned B. V. P e r f i l i e v 's method for preparing microtome sections from slime. This method may be applied to finely granular soils as well and, in its turn, gives new data. Finally the extension of W i n o g r a d s k y 's method to the study of the soil Protozoa should be noted. Protozoa, being dyed as usual with erythrosine, generally do not give a good picture. Better results have been obtained as shown by D o g e l (1927), by the use of eosine. There is too little a number of Protozoa in poorly or moderately cultured soils, to be seriously studied in this way, but for highly cultivated soils it may be of some importance.

2. Isolation of microbes, methods for the study of biological activities, soil and other cultures

Accumulative cultures, isolation of microbes, calculation of the biological activity and soil cultures (spontaneous cultures of W i n o g r a d s k y, W a k s m a n 's method, etc.) play an essential rôle in the study of microbes in soil and of their activity. The basic line of approach to all these methods is the utilization of the principle of elective cultures, grounded and developed by W i n o g r a d s k y. Yet at their putting into wide practice, the following difficulties were met with in our Union.

¹ L'Italia Agricola, 4 (1928); Nuovi Ann. dell' Agricoltura (1927); Jul. S t o k l a s a 's Festschrift, 1928.

To begin with we had to note that when adding some one substance or modifying the dosage of some one factor, we had not the right to affirm that only this factor had changed hereby. For instance, when adding mannitol to soil, according to Winogradsky's prescription, not only an energetic substance is added to soil but there takes place a series of most serious shifts — fall of the pH, fall of the rH, transfer of ferrum into solution and many others, the action of which is not weaker, sometimes, than that of the «only» change of situation taken into account. This has served to peculiarly bring to the front a thesis I have always emphasized — that of the importance of an immediate determination of the respective changes in the experiment. Such a determination being absent, the comparison of certain soils with others is to be entirely excluded—owing to the multi-formity of our soils and of other conditions. One has only to bring to mind errors committed in non-detecting the *Azotobacter* in many soils, where, as a matter of fact, it is to be found (E. E. Uspensky, 1930; Novogradsky, 1932).

Further on, one often makes errors, even when correctly evaluating the rôle of some substance, in choosing its concentration. For instance, at our giving, after Waksman, 300 mg. of NH_3 per 1 kg. of soil, when we calculate nitrification we put different soils into entirely different conditions: for some they do not seriously differ from those in field, for others most pronounced changes are observed to take place. Indeed, in evaluating soil possessing a high buffering capacity relatively to acidity, an artificial raising of the NH_3 concentration is of no danger; the buffering capacity being low, on the contrary, there takes place a marked shift of the reaction towards acidity, especially so around nests of nitrifying bacteria (N. V. Yashnova, 1930). Therefore, the introduction of lime, in the latter case, cannot be considered as an activation of the dormant nitrification. Such concentrations of ammonia do not occur in podzols under field conditions, and nitrification does not retard other processes. We should welcome in that respect the work of Ziemiećka, who has also lowered the doses of ammonia salts¹.

We ought to endeavour, in such tests, to take deeper account of the inner peculiarities of the soil conditions in connection with the distribution of microbes in the soil. Thus, we have shown (E. E. Uspensky, 1930) that even the introduction of lime, in tests with nitrification after Waksman, does not solve the problem of the neutralization of acids as an excessive acidulation is formed in the centres of nitrification in virtue of the different mobility of NH_4 and CaCO_3 . Therefore the determination of nitrates after a lapse of 21 day only embroils the matter and cannot be considered as a study of biological activity if obser-

¹ Roczniki Nauk Rolniczych i Lesnych. XXI (1929).

vations are made as to what is taking place in soil, what are in it the alterations of the microflora, and so on.

Yet not only the disposition and other peculiarities of microbes should be taken into account in such tests. The purely soil complex should also be most thoroughly studied. Thus Winogradsky¹ has been recently disappointed in mannitol and suggests replacing it by benzoates. This prescription is not suitable for our podzol soils, — neither is that of Loew² with sodium acetate.

Soil being unsaturated, the cation penetrates into the soil complex, and there is created a marked acidulation by the addition of sodium acetate, as well as by that of salts of the benzoic acid, the constant of the dissociation of which is still higher.

Finally, not wishing to produce artificial changes, we must not imagine simple outward imitation of nature to be the easiest way to attain it.

We have the right, and should profit by it, of modifying natural correlations in our experiments, yet we must take them into account and learn how to evaluate the natural complexes in the sense of their inner interdependence. Thus, aeration is a very essential moment in microbiological cultures. Yet, one should not think that, when placing the culture on the upper surface of a silica gel plate, we always create optimal conditions for an aerobe.

First of all the aerobic capacity of microbes varies in connection with the microbe being well or ill fed and, consequently, in connection with the composition of the medium³. Secondly, the oxidizing conditions of the medium itself are not a simple function of the air-access, it is necessary to take into account the oxidation-reduction potential and related moments. Finally, alterations, occurring at the same time, may have some other noxious effect on microbes. For instance, according to the soil, to the tempos of the development of the studied microbe or its «satellites», a full access of air either entirely oxidizes iron and throws it out of the sphere of action or, on the contrary, oxidized and more poisonous iron goes over in excess into the solution due to the formed acids or to some other complex.

The initial state of microbes in soil plays a very essential rôle for their isolation. A microbe being overfed with iron and on the verge of ruin, if put under conditions bringing to a still greater accumulation of iron, may perish. The same may happen, if a microbe famishing from a deficiency in iron or in some other substance, is put at once under optimal conditions for rapidly growing forms. In that case the microbe, before recovering, may perish from an excess in some substance. We had to primarily investigate all this upon aquatic organisms. But, as it was shown by

¹ Ann. Inst. Pasteur. 1932.

² Zentralbl. f. Bakt. II Abt. 1927.

³ Z y c h a. Archiv f. Mikrobiologie. 1932.

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our experiments, with the addition to soil plates of agar-agar and of silica gel (see below, VIII), the corresponding moments are very important even for the isolation of the aerobe *Azotobacter*. Therefore, even though there be much *Azotobacter* in soil, it may produce no colonies if our line of approach will be too simplified. Yet, if we add to the same soil some *Azotobacter* taken from a culture in another condition, it may produce colonies.

Proceeding from the same considerations we should not be too much afraid of liquid media. If we regulate all other correlations, they do not prove to be an unconditional «swamp», sometimes we observe in them seemingly paradoxical correlations. Thus, in some of the Fergana soils, in certain horizons, nitrates are better formed at 90% than at 40% of full water-capacity, experiments being carried out after Waksman.

It is not casual, beyond doubt, that when selecting material for illustration we choose every time combinations of physicochemical factors, microbes acting as oxidizers, and the like.

However, the correlations are more complex. It is necessary, on the one hand, to take into account the cycle of development and form (see above p. 42) of the microbe analysed, on the other, the purely biological correlations with other microbes also (bacteriophagy, etc.). We are now only approaching the study of the mentioned correlations (Novogrudsky, 1930). Only serious work in that direction may give a really microbiological method. Certainly, we cannot consider as correct those lines of approach when, in studying the importance of biological moments, the authors detach themselves from the actual milieu and overvalue observations in a hanging drop, etc. We find this to be a pseudo-classical style, which does not take into account either the newest achievements of the immediate study of the physicochemical surrounding, nor the recent achievements of the physiology of the interaction between organism and medium, disagreeing with the theory of evolution, which was not created in a hanging drop.

At the same time, we do not deny any of the above enumerated ways and, in particular, consider observations on separate cells to be of an extreme importance. But we presume that the method of microbiological research should develop in connection with the peculiarities of those natural-historical objects, in which microbes enter as a part. Further on, a biologist cannot be satisfied with physics and chemistry of the past century and should, on the contrary, set forth new problems for biochemists, physiochemists, etc., as Dutrochet has done in his time with osmosis. Finally, he should not detach the study of the inner moments of the life of the microbe, from the study of the microbe itself in all the multiformity of its interaction with the outer medium.

Therefore, our platform in the elaboration of the method for studying the distribution and activity of microbes, was based on:

1. an immediate connection of moments described above with the study of the soil in the field;

2. on the study of the distribution and activity of microbes on the basis of an endeepened study of their physiology (see below, chapter D).

3. Study of the type and dynamics of microbiological processes in soil in the field.

The most essential moment in the study of microbiological processes in the field is that of the elaboration, for each individual case, of complexes of physico-chemical and biological observations necessary in it.

The wish is, certainly, natural, to embrace a possibly wider circle of observations, yet it is impossible to treat too many subjects at once. A correct choice of ingredients and term limits for determining them could be effectuated but on the basis of a great preliminary work. Rules for the taking of samples in connection with soil profiles, condition of soil, and its physical and chemical peculiarities had to be studied for individual regions and soils. All this must influence the choice of method.

It stands to reason that we cannot dwell upon all details and our purpose is only to indicate the essential schemes which had been worked out in a greatly resembling form, on the one hand—by the microbiological division of the Scientific Institute on Fertilizers (Uspensky, 1929), on the other — by the Institute of Agricultural Microbiology of the Lenin Academy (Korsakova, 1930).

The necessity should be noted, first of all, to distinguish whether the soil type is to be established in connection with local practical problems, or stationary observations, next in turn, are to be carried out. It does not, certainly, mean that both types of investigation are completely separated. Just on the contrary, only stationary observations, establishing the dynamics of some process elucidate the type to the end, certain features standing out with particular relief only at the application of fertilizers. For instance, the peculiarities of the absorbing complex of grey forest soils of the Nizhni-Novgorod region are clearly manifested in a feeble action of calcium carbonate and a strong action of calcium hydroxide; yet the primary orientating investigations should not be mixed with current observations. As purely soil investigations in our country are always well provided for, I am ignorant of any notable works conducted without the determination of the type of soil and of its horizons.

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Our microbiological researches usually tend first of all to supplement this type and, further on, to show — by the selection of the complex of stationary observations, — the variations of this typical picture in connection with meteorological data of the year, with tillage, with culture and fertilizers. Besides, we find it impossible to tear off transformations of nitrous substances from the action of phosphorus compounds. For instance, it is usual in chernozem (Nossovka, T. N. Germanov) for the action of phosphorus to be either positive or negative, according to nitrification having, or not having accumulated nitrogen. The addition of ammonia salts to markedly carbonatic serozems of Turkistan (S. I. Kusnetzov, see this volume), and a subsequent rapid nitrification, bring about an increase in the amount of soluble calcium and also, following it, a precipitation of the already insignificant amounts of phosphate. That is, the acid formed suffices for dissolving a certain quantity of calcium carbonate, but is insufficient for noticeably shifting the reaction. In consequence, the physiological and microbiological acidity of ammonia salts causes but a decrease in soluble phosphorus and a lowering in yield.

It is possible to specify the variations of the soil in parallel lots of land by methods of microbiology; valuable data are given thereby by the method of soil plates (plaques) with drainage (see p. 54 and 61), in respect of the characteristics of the absorbing complex of the phosphates and of potassium mobility and relatively to iron as well.

Further on, neither stationary observations nor those orientative can ignore the other processes of the nitrogen cycle, first of all — denitrification, even were the problem set only in relation to the enriching with nitrogen. Denitrification should be specially taken into account at watering, and it would be even of great expedience to work out the schemes of watering and of addition of fertilizers to the soil, proceeding from a study of it in such cases (see S. I. Kusnetzov).

The course of the accumulation of nitrates, in its turn, cannot be understood, unless ammonification has been taken into account. As a definite minimum, we usually apply in practice the following complex of microbiological and chemical determinations.

A determination of the number of bacteria according to the principal groups in preparations after Winogradsky's method with Germanov's modification. A determination of the number of denitrifying and ammonifying bacteria — after Hiltner. Besides, according to the prevailing forms in the given soil, attention in stationary observations is concentrated on denitrifying bacteria, utilizing either calcium citrates or tartrates, or cellulose.

As to chemical determinations, that of the amount of nitrates and nitrites is not sufficient for the study of denitrification.

It is necessary to determine the watersoluble organic matter after K u b e l, the pH (compare p. 59) and in many cases the rH. Apart from this, K o v r o v t z e v a has elaborated a method for the determination of the gaseous nitrogen of denitrification.

W a k s m a n's method for studying nitrification does not give, under our conditions, a full conception (see Y a s h n o v a) of the real course of nitrification in the given field. Yet it is most valuable for the study of correlations in soil between different microbes and for elucidating possible shifts. We are inclined, therefore, to utilize it not so much for stationary observations, as for the elucidation of correlations in soil for the whole cycle of nitrogen transformations. Naturally, the whole complex of determinations is to be repeated.

The numbers of *Bac. mycoides* have been chiefly determined for the study of ammonification up to most recent times. Yet *Bac. mycoides* is isolated with difficulty in cotton plant regions, especially so in winter. The study of the problem of its different forms, as well as that of its antagonistic action towards soil actinomycetes (see B o r o d u l i n a), is necessary to be brought to an end for these cases. Besides watersoluble ammonia, adsorbed ammonia must also be determined.

For making clear the picture of nitrogen fixation, direct chemical determinations give good material but for one season and only in cases where the total amount of nitrogen is relatively small, and nitrogen fixation takes its course rather energetically (for instance, the Turkestan serozem). We consider the determination of total nitrogen necessary to be carried out in podzols at least once a year for bringing into concordance the observations of many years. The counting up of the *Azotobacter* in microscopic preparation is checked by the germination of preparations after A. S. R a z u m o v (see p. 45), by spontaneous cultures, as well as tests with silica plates. Yet, a decisive significance cannot be attached to the latter (see N o v o g r u d s k y; N o v o g r u d s k y and N a u m o v a).

The application of silica plates for studying nitrification is but now being started in our Union. W i n o g r a d s k y's method gives good results with soils that have been well fertilized. Matters are worse with less cultivated soils; respective methods are being worked out.

The dates and frequency of observations play a most important rôle in stationary observations. Data of the Scientific Institute on Fertilizers have shown that analyses carried out rarer than once in two weeks may only bring confusion. In transitional periods dynamics may be spoken of only if more than ten tests are made.

Microbiological analyses determining nitrifying and denitrifying bacteria should be carried out directly after the sample has been taken. After it has dried, it is of no use to speak of quantitative determination of the activity nor even of the preser-

vation of this or other microbe. We may certainly isolate this or other microbe also from dried up samples, but we cannot warrant its absence if it has not been isolated. The *Azotobacter* stands drying better; this has already been noted in literature.

The determination of the total number of the saprophytes on gelatin and agar-agar plates is not included in the usual programme of stationary observations, but is expedient for the determination of the degree of the soil's culture. The same is probably true of the determination of the number of the *Protozoa*, but we do not dispose of sufficient material to affirm this. For specially determining the degree of the lack of culture of a soil from under forest, the number of mould fungi is not deprived, seemingly, of a certain importance. Good results in the determination of their number have been lately obtained by the application of N. G. Ch o l o d n y ' s method (see p. 46) with glasses dug into ground.

VIII. Estimation of the soil's requirements in fertilizers.

One of the main problems, when studying the distribution of microbes in soil, was to elucidate soil biodynamics and thus to contribute to measures taken by agronomics. The most important lines of approach in that direction and the current problems have been noted above.

Yet, the application of the soil evaluation according to the growth of microorganisms has already outgrown the limits of making observations with the only purpose of explaining biodynamics in the field and has brought long since, if it may be so expressed, to micro-pot-culture experiments. The essential idea in that direction had been expressed so far back as in 1869, by Raulin¹, in his classical work on the physiology of fungi nutrition.

It does not enter our problem to examine all the literature on that topic. It is but recently that we have returned to that problem, namely after the I International Congress of the Society of Soil Science in Washington, in 1927. Soviet investigators have utilized in their works the *Azotobacter*, brought forward for such purposes by Christensen² and Winogradsky³ (see Issakova, 1929; Kriutchkova, 1930; E. E. Uspensky, 1930; Minenkov, 1932; Simakova, 1932) as well as *Aspergillus niger* (Butkewitsch 1932; Simakowa, 1932) recommended by Raulin, and which had, since long, been studied in that

¹ Raulin. Etudes chimiques sur la végétation. Thèses. Nouvelle édition. p. 209, Paris. 1905. See also — Ann. Science Natur. (5) XI, 93, 1869.

² Zentralbl. f. Bakt. Abt. II, 1907.

³ S. Winogradsky et J. Ziemiańska. Ann. Inst. Pasteur XVII, 36—62, 1928.

respect by W. S. Butkevitch¹. Azotobacter tests were applied, yet, the following modifications were introduced into it, after the shifts, occurring in different soils at the addition of mannitol, had been investigated by A. P. Kriutchkova.

Additions of the standard culture were applied in connection with the fact of the Azotobacter not being detected in all the soils investigated, and its absence in just the soils that had to be subjected to liming, as well as to our investigation. The same modification had been introduced into Winogradsky's method, independently of us by Sackett in America², approaching in fact to that of Christensen.

In our experiments Azotobacter was not only added to the investigated soil, but it was added in definite large doses (Kriutchkova — 2 000 000 cells per 1 g. of soil), reckoning to transfer the centre of gravity of the estimation, in the case of phosphorus, not on the stores of soluble phosphates, but on the readily mobilized phosphorus (E. E. Uspensky, 1930), it being found possible, besides, to reduce the time assigned for the test to 24 hours.

Further on, drainage was introduced (Kriutchkova, E. E. Uspensky — 1930), in order to obviate the lowering of the pH and the rH when adding mannitol, especially in northern soils.

Sackett did not resort to that method, inasmuch as he had to deal with more alkaline soils. This may have conditioned failures at the application of this method to soils of Iowa recorded by Keller³. As observed, the lowering of the pH brings about, among other things, an increased need in calcium and a decreased one in phosphorus.

Later on in the process of work with different soils, the necessity became evident of supplementary modifications.

In some soils an excess of iron seemingly inhibited the Azotobacter's growth. In order to decrease its active concentration extra portions of agar-agar or silica-gel were added in quantity of 0.1 of the total amount of water (E. E. Uspensky, A. P. Kriutchkova and U. G. Oxentian⁴).

Besides, new schemes were suggested for investigation, according to the next problems having to be solved and to the degree, to which the region in question has been investigated. The so called scheme II (E. E. Uspensky, 1930) has been calculated for less investigated regions. It records tests of the soil investigated after different additions of lime (4; 2; 1; 0.5; 0.25; 0.125% CaCO_3) or phosphates (1.5; 3; 4.5; 9; 18 mg. P_2O_5 per 100 g. of soil), or potassium (0.01; 0.005; 0.0025; 0.000625; 0.00031% K_2O).

¹ Journal of Exp. Agronomy, X, 136—141, 1909 (in Russian).

² Colorado Agric. Coll. Exper. Stat. Bull., № 375, 1931.

³ Zentralbltt. f. Bakt. Abt. II, 1932.

⁴ Transactions of the Inst. on Fertilizers, № 108, 1933, Moscow.

Thus, with one standard field, the quantitative need in fertilizers may be established.

W. S. Butkewitsch has also introduced supplementary modifications into the method with *Aspergillus*, as compared to his old modes of investigation and those of H. Niklas. The most important of them is his having replaced *Aspergillus niger* by the less acid *Aspergillus Oryzae*. Such a substitution, eliminating the excessive dissolving capacity of the *Aspergillus*, simultaneously creates difficulties, as it requires a sterilization of soil.

The chief mass of investigations in our Union has been carried out with the *Azotobacter*. I, personally, prefer it to *Aspergillus*, not only in virtue of technical considerations, but also in principle, due to the following. When working with typical soil organisms, we do not attach to our research a narrow-evaluating character. We do not determine at the dosage of the quantity of the *Azotobacter* the stores of this or other substance, but its activity in the course of time, i. e. we actually utilize biology, as we have to do with organisms; these latter, unlike a reagent are not wasted, but grow and act in the course of time. We, generally, consider that our respective methods should compete with chemistry not in the line of simplifying operations, but in that of discovering new possibilities.

Since we utilize the organism of a good soil, being in itself an object for the influence of fertilizers, we may not only estimate soil within definite limits of the known (there are enough methods for this), but plan, at the same time, new problems to be solved. Thus, for instance we see that phosphates in some soils remove the excess of iron, besides their other activities. Further on, our laboratory in Turkestan has established¹ that ammonium sulfate often does not produce any effect on the yield as it makes calcium go over into solution. This, on the background of great alkalinity, does not result in the dissolution of phosphates, but, on the contrary, precipitates them. A perfectly new local problem inaccessible to *Aspergillus* has been solved in that way, a serious corrective entering our conception of the importance of acid and physiologically-acid fertilizers.

Under conditions of the uninterruptedly growing technics of the socialistic construction, the so-called store of fertility of Mitscherlich does not play any preeminent rôle. Yield is markedly changed dependently on tillage. To elaborate a quasi «micro-Mitscherlich» would be of small significance under the said conditions. Of so much greater an importance would it be to not only evaluate, but, by means of the microbiological estimation, to find out the cases of a feeble or strong influence of fertilizers. This is possible to be achieved with the *Azotobacter*, but not with

¹ See S. I. Kusnetzow's work, this volume, page 113.

Aspergillus. Thus microbiological evaluation and microbiological diagnostics come nearer one to the other (see above), which is not difficult to understand. Instead of two different problems — explanation of the soil dynamics and substitution of the chemical estimation of the soil supplies — we have but one problem to solve: control of biodynamics for the raising of yield.

When the problem is thus formulated, microbiology should not be afraid to compete with chemistry, soil dynamics being biodynamics. Therefore, a rational microbiological estimation is not one of the type of *Mitscherlich*, not even symptomology only, but the revaeling of inner correlations and a way for control.

D. General conditions of the life and work of soil microorganisms

I. Temperature

As we had noted (chapter B II, page 19), when exposing the achievements of biochemistry in the domain of soil microbiology, it should have been our duty to elucidate as well the general conditions of the course of these or other processes and their energetics. We had also pointed out there and then that the development of a whole series of similar investigations had been stimulated by observations on the spread and work of microbes in soil. Besides, the behaviour of microbes is connected not only with the physico-chemical conditions of the course of separate processes but also with their range of adaptability. This is what made us postpone the examination of respective problems, it being understood that we shall return to them as soon as we have studied the spread of microbes and the totality of problems connected with it.

One of the most general conditions for microorganisms to exist and work, is a suitable temperature.

We have to note, in this direction, two essential series of researches for the elapsed period. The first is constituted by *L. Rubentchik's* researches in Odessa, he experimented in connection with the work on the fields irrigated with sewage waters, and has given much material, in particular, as to the work of the soil microbes and at low temperature (*L. Rubentchik*, 1927).

Secondly, we have a series of investigations carried out by *E. N. Mishustin* (1925; 1926; 1932) who has endeavoured to establish the dependence of the cardinal temperature points of different strains of organisms on climatic conditions of the place of their origin.

II. Osmotic conditions of the medium

Much attention was given to clarify the osmotic conditions of the medium inhabited by different microbes.

Works of L. Rubentschik (1928; 1929) and E. N. Mischustin (1933) have again to be noted in this field. Rubentschik has established that nitrifying bacteria may carry out work at a very considerable salinization.

He has shown the same to be true for microorganisms decomposing cellulose and sulfate reducing microbes.

Ginsburg-Karagicheva has utilized the different adaptability of different strains of sulfate reducing bacteria to high concentrations of salts for distinguishing between microbes living in the depth of the Earth's crust (1000 m.) and those living near the surface, which could have entered it as an occasional contamination.

E. N. Mischustin, in his joint work with M. Messineva, tends to connect the osmotic stability of microbes with climatical conditions of the place of their origin. According to his data, the strains of *Bac. mycoides* of a more southern origin, are adapted to a more arid climate and, connected with this, to a greater osmotic pressure of salts.

It should be noted, besides, that data of a series of authors (Keller, Germanov, Sabinin, Henkel) point out the good growth of *Azotobacter* under conditions of considerable salinization¹.

It stands to reason that phenomena of the so-called antagonism of salts, the possibility of plasmoptis and so on, should be taken into account in studying the influence of high concentrations of salt. Works of P. P. Smirnov (1926) partly fill up this gap.

III. Acidity

At the present time no biochemical or physiological work may be done without the active reaction being taken into account. Yet in the pre-war time almost all of the scientists were regardless of it. All the mass work in that direction has been performed after the revolution.

In our Union one of the first to introduce the determination of the active reaction and to take up problems related to it, was our microbiological laboratory of the Moscow State University. We approached that problem in two ways: first of all we took into account the essential methods and trends being developed in the biological laboratories of the West. Yet, our having originated from the Timiriazeff laboratory did not allow us of limiting by this our

¹ See also the report of T. N. Germanov in this volume, p. 123.

investigations and made us look for more physiological lines of approach in western literature, as well as to find them by our own means. However strange it may seem, many and many important physiological and biochemical schools did not manifest of any haste in that respect, and we, being just beginners-physiologists, had almost to lead a struggle for that trend. Our colleagues valued in the pH, first of all, a certain new factor of the type of temperature which they discounted in most cases, without looking into the substance of correlations. Of this substance, the very little that was known was connected with the changes of colloids. For us, on the contrary, the pH and since 1916, after Reed's¹ work, the oxidation-reduction potential were of great value as being a striking instance of an immediate entering of the so-called medium conditions into the equation of the process of reaction. C. A. Timiriazeff has given in his Croonian lecture a vivid picture of the transmission of energy from sun to plant. The thesis he had established, as to there being created in a chlorophyll grain a high tension of energy, which explains how the decomposition of carbonic acid is made possible, could be well applied in the domain of microbiology.

All cases of the chemosynthesis, established by Winogradsky, are connected with the utilization of a high tension chemical energy. Therefore, this problem had to be carefully examined. Yet, how were the corresponding tensions to be measured, and in what could they be manifested? These were the problems we hoped to come nearer to through the study of fluctuations in the pH and in the oxidation-reduction potential.

One had to know, however, how to distinguish between the action of the pH, let us say, telling on this or other process directly, and the alteration in acidity following, the dissolution of iron, of calcium, or of some other substance, when the latter begins to act². It is perfectly obvious that not every microbiological process offered opportunities for its study in that respect, as for instance, microbes of both phases of nitrification require relatively a great deal of iron³. This excludes, when working with them for the maintenance of a definite pH, the use of phosphate buffers⁴ as the sedimentation of iron by phosphates strongly shifts the alkali limit towards that acid. Winogradsky⁵ correctly explains in his recent work the slowness of growth

¹ Botanical Gazette, 1916.

² E. E. Uspensky. Iron, as being a factor of the distribution of algae. Trans. of the Bot. Inst. of the Moscow Univ. pp. 25, (1925), etc., as well as E. E. Uspensky (1924) and E. E. Uspensky, Eisen als Verbreitungsfaktor et.. S. 25. Jena. E. Fischer. 1927.

³ E. E. Uspensky. Manganese in plant. I. Journ. of Exper. Agron. (1915—1916); Manganese in plant. II. J. of the Mosc. Section of the Russian Botan. Ass. (1922).

⁴ E. E. Uspensky. Iron etc, pp. 29 and 30.

⁵ Ann. Inst. Pasteur. L., 344—432 (1933).

in Gaarder and Hagem cultures. His carbonate mixtures fully correspond to our former observations, published in connection with the algae cultures. Yet, doses of iron to be given under such conditions are so great, poisoning may easily occur at adding acid. Therefore, the acid limit is found to be shifted towards that alkali. This way is not suitable for solving our problems, neither for conceiving correlations in a field surrounding. Besides, the relationship to the pH is greatly dependent on the total good feeding and on the content of nitrogen, problems not to be simply solved in respect to cultures of nitrifying bacteria as yet. The latter, even more than the *Azotobacter*, require Gainey's¹ method to be followed, i. e. the accumulating of a mass of data for different plots of land, for different soils, cultures, and so on. Authors cited in Winogradsky's latest work, have all endeavoured to perform this; yet new difficulties have arisen, because of an insufficient knowledge of species composing the group. Therefore, when accumulating the respective material, we did not make large scale experiments for elucidating the acid-alkali limits, neither with nitrifying bacteria nor with the *Azotobacter*.

Our laboratories have given much more of their attention in this domain to denitrification. Denitrifying bacteria are «afraid» of heavy metals, being actually satisfied with traces of iron, in consequence of which work with them is rather easy. Denitrifying bacteria get stronger at an excess of liming², due to which practice required work with them to be carried out, in respect to pH in connection with liming. There were also theoretical premises for establishing an inner connection between the pH and the process itself. Finally, the application of the pH to denitrification seemed to have explained the paradoxal results of S. T. Naguibin³ which was a good pledge for further success. It proved at once possible to explain the difficulties with the culture of denitrifying bacteria. A lack of conformity after application of prescriptions with water taken from the public water-supply

¹ Gainey. Proceed and Pap. I Intern. Congress of Soil Science, 1927.

² Arndt. Landw. Jahrb. XXIX, 191—213, 1916, LL, 197—319, 1918.

³ Nothing but a short note has remained of S. T. Naguibin's work, which may be seen in the «Protocols of the Moscow Soc. of Investig. of Nature» 1915, pp. 134—136 (Protocols are annexed as supplement to XXIX volume—1915, Bulletin Soc. Nat. Moscow, 1916). Naguibin demonstrated that at his replacing the filtering paper by hygroscopic wadding, denitrifying bacteria did not ferment it. Besides, quite incomprehensible was the fact that the process with KNO_3 terminates earlier than with $\text{Si}(\text{NO}_3)_2$. The very first of my experiments have shown denitrification to be retarded with KNO_3 due to leaching, and wadding to be of no hindrance when using alkali of phosphate. In 1924 we had fully put into practice the determination of the pH (W. I. Terebikhina-Uspenskaya, P. D. Varlyguin, E. M. Dmitriev). In 1920, in connection with work started at the Institute on Fertilizers I entrusted T. M. Zacharov with thoroughly elaborating this subject.

got to be simply explained by diversities in alkalinity and in the buffering capacity of water, taken from different systems of water-supply. The difference between the Moscow and Leningrad experiments was thus elucidated. Having resorted to cultures in media of a definite chemical composition we could proceed to further experiments. Fundamental tests were carried out by T. M. Zacharova (1923; 1925; 1929); it was also she who made the corresponding observations in field conditions. T. M. Zacharova has established the optimum of the pH to lie between $\text{pH} = 7-8.2$, for denitrifying bacteria disintegrating the cellulose and for bacteria of the group *Bac. Stutzeri*. Denitrification rapidly decreases from the neutral point towards the acid limit ($\text{pH} = 4.6.1$) and practically stops at $\text{pH} = 5.5$. It stands alkalization up to $\text{pH} = 9.88$.

T. M. Zacharova's data were confirmed later on by Kovrov'tzeva (1927) and M. P. Korsakova (1929); Salimovskaya, when working with denitrifying bacteria oxidizing sulfur, obtained approximately the same curve.

The establishment of these correlations is of interest not only because it gave certain indications as to how should soil be limed, and towards an explanation of the shift of processes in field, but also because it allowed to closely approach the problem of the physico-chemical correlations of the very mechanism of the reaction, as well as that of an immediate connection between influences from without on processes within the organism. Reduction of nitrates, as it is well known, takes its course better in an acid medium. Denitrification, better occurring in an alkali medium is, seemingly, connected with the fact that the main difficulties lie in the activation of the organic matter, which gets oxidized. Therefore, the centre of gravity in the study of transformations should be brought nearer to the molecule of the organic matter. It is here we should endeavour to detect intermediate products, instead of looking for various degrees of the reduction of nitrates (see below p. 62).

For obtaining a clearer conception of the shift of the processes, it should be most essential to study the changes in the pH within bacteria cells.

Besides denitrifying bacteria, pH was more thoroughly studied in this country than in the West — in connection with urea decomposition (Kusnetzow, 1930). It had an immediate interest in connection with the rôle of the peat litter, eliminating the loss of ammonia. S. I. Kusnetzow established the acid limit to lie — for *Urobacillus Duclauxii* at $\text{pH} = 6.6$; for *Urobac. Macdouxii* at $\text{pH} = 7.0$ and for *Urob. Pasteurii* about $\text{pH} = 8.1$. The optimum being for *Urob. Duclauxii* at $\text{pH} = 7.4-7.7$, for *Urob. Macdouxii* at $\text{pH} = 8.2$.

We have already noted that no detailed experiments have been made in this country with the *Azotobacter* for elucidating its limits of the pH, which, according to all data, show a large range, not only in connection with its race, but also with the complex of the medium conditions. Yet, with the *Azotobacter* the first observations were made on the connection between its attitude towards aeration and the acidity of the medium (E. E. Uspensky, 1923, 1930). With increasing alkalinity the *Azotobacter* markedly shifted towards anaerobic conditions. This observation was, later on, fully confirmed at the study of alkali soils of our South-East region (Richter—1928; Sabinin and Henkel—1927; Henkel and Zacharova—1930; Sabinin and Minina—1932).

It is curious to note, according to S. I. Kusnetzow's data, that in alkali soils of the Gandzha Experim. Station, even after watering, the rH in soils is found to be near to 31¹. This may explain the behaviour of the *Azotobacter* which, in spite of its aerobity, may be met in Turkestan at the depth of 13 meters. These data were one of the reasons for prompting a more detailed study of the oxidation-reduction potential, in connection with the life of soil microbes.

IV. The oxidation-reduction potential.

The following of the achievements reached in this field may be here noted.

The degree of the aerobity of the *Azotobacter* was studied in greater detail (in our soils it does not grow at the rH being lower than 24), due to which the method of soil plates could be improved (Kriutchkova, 1930).

M. M. Cononova established (1932) the way for struggling against denitrification, under conditions of Turkestan conducting watering, on the study of the changes in the oxidation potential of the soil.

S. I. Kusnetzow (1930) not only established urea decomposition to be possible at a low rH (from 28 to 0.8), but also showed it to be the way for effecting denitrification with the assistance of the urea bacteria.

Contrary to expectations in the so-called «direct denitrification» with the assistance of *Bac. denitrofluorescens*, the rH of the medium does not fall very low (observations not yet published by A. P. Kriutchkova, Korochkina and Ravina—rH=22), even in the conditions of an almost entire decomposition of nitrates (0.5 mg. out of 35—36mg. N per 1 litre), which corresponds to former observations (T. M. Zacharova, 1923; Kovrovitzeva, 1927) of deni-

¹ See this volume, p. 113.

trification as not being at all a strictly anaerobic process. The comparison of these data with Clark's¹, Cannan's and Cohen's (1926) experiments with the *Bac. coli*, which brings reduction only up to nitrites, will make it obvious that the mechanism of the liberation of free nitrogen is far from being bound with a peculiarly energetic reduction. We are inclined to think that true denitrifying bacteria differ from those of the type of *B. coli* by the fact that they themselves readily form amides, while in the case of *B. coli* these have to be added to the culture medium.

This corresponds to the general rule² that amide organisms are more alkaline and that the formation of amides requires oxidation. *Bac. coli*, being an acid organism, is not one amide neither do the anaerobic conditions of its existence correspond to the formation of amides³.

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The «Bureau of Agric. Microbiology of the State Institute for Experimental Agronomy» and the «Institute for Agricultural Microbiology» are two consecutive names of one and the same institute founded by S. P. Kostytshev. The Proceedings of the Microb. Institute, previously to their own organ being founded, were published in a general organ, «Annals of the Institute for Experim. Agronomy».

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MICROBIOLOGICAL EVALUATION OF THE SOIL'S REQUIREMENT IN FERTILIZERS

E. E. USPENSKY and A. P. KRIUTCHKOVA

Introduction. History

The notion of the possibility of microbiological evaluation of the soil's requirement in fertilizers was advanced very long ago. It was first formulated by R a u l i n (1869) in the latter's classical work on the physiology of nutrition of mould fungi.

«Tous les agronomes reconnaissent l'insuffisance de l'analyse chimique appliquée à l'agriculture; c'est pour ce motif qu'on a proposé d'analyser les engrais et les terres par végétation. Or, s'il est vrai que, au point de vue chimique, les Mucédinées se rapprochent considérablement des végétaux supérieurs, elles offrent à l'agriculture une méthode d'analyse qui par la rapidité d'opération, par la facilité d'exécution, par les nombreux éléments chimiques qu'elle peut atteindre, par sa précision, est incontestablement supérieure à la méthode d'analyse par les grands végétaux fondée sur le même principe».

But R a u l i n's time did not take advantage of his idea. In the first place, microbiology had not entered into close contact with agronomy, and secondly, in such cases where it did occur, other more direct questions were next in turn, as, for instance, the problem of root nodule bacteria, of nitrification, of bacterial losses of nitrous compounds, etc. Furthermore, though R a u l i n was able in the lower plants to ascertain nearly to the end the list of necessary elements and other like questions, for higher plants the position was somewhat different — the complexity of the environment did not allow an immediate approach to the establishment of quantitative relations between the yield and the presence of one or the other nutritious substance. Moreover, the greater complexity of the higher plants and the economical magnitude of their culture in general, did not allow R a u l i n's point of view.

Only at the outset of the 20-th century agronomy, having solved a series of basic questions of plant nutrition, began to study energetically the modes of approach for establishing quantitative relations between the yield and the presence of nutritive substances.

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At that time was again raised the question of microbiological evaluation of the need of plants in fertilizers. In this, our compatriot W. S. Butkewitsch (1909) literally advances in the footsteps of Raulin. Together with Koszelezky he uses cultures of *Aspergillus niger*. It is a great pity, that Russian reality of that time was but little favourable to the development of scientific agronomy. The studies of Butkewitsch and Koszelezky found no response in our country and remained unfinished. It seems, that those studies were also little known in the West, and they begin to be extensively quoted only after the works of Benecke and Söding (1928).

Simultaneously with W. S. Butkewitsch, and even somewhat earlier as to date of publishing the first paper, Christensen (1907) began his studies in this direction in Denmark. It is known that Christensen in a series of works (Christensen and Laarsen, 1907, 1911, 1915, 1916, 1922), approached the question somewhat differently. He advanced first of all from the field, and his organism — *Azotobacter chroococcum*, is an organism of good culture soil. Christensen first of all studies the spreading of the *Azotobacter* in various soils, notices how it avoids the sour soils grown over with *Rumex acetosella*, combines the characteristics of the soils, contrasting those data with samples for effervescence from HCl, etc.

In this case Christensen established the presence of *Azotobacter* sowing some soil or other into the nourishing substance containing all that is necessary for the *Azotobacter*.

Besides this approach, differing as to principle from that of Raulin — Butkewitsch, Christensen also carried out a series of experiments of their own type. Soil (as a source of nutritive salts) was added to a mannite solution and a standard culture of *Azotobacter* was sown over the whole of it. Reports were put up in parallel; into them lime was added. In the case when the growth of *Azotobacter* was observed only with an addition of lime, it was possible to assert that the soil needs lime. Somewhat later Christensen began also to determine in the same way the requirement of soil in phosphorus. Those series differed from Raulin's approach in two ways. The use of the typical soil organism was a great advantage. Nevertheless, as Christensen remarks himself, the calculation of the yield is more subjective, as it is based not on weighings but on the evaluation of the *Azotobacter* film by eye-sight, which, generally, varies rather strongly.

Experiments by Christensen, carried out by the latter together with Laarsen, were extensively set up and found practical utilization. 28 562 samples from various parts of Denmark were inspected up to 1916.

The studies of Christensen found a warm response in H. Niklas, from Bavaria.

Here the same experiments with *Azotobacter* were combined with chemical study and were extensively set up. The map of Bavaria establishing the need in lime and phosphorus for different districts was provided, and the scheme for studying and mapping the separate farms was worked out.

It is very natural, that working on soils less podzolised or leached, than those of Christensen, Niklas paid a special attention to the need in phosphorus, and in this direction he seriously developed and more deeply investigated the approach of Christensen.

The new ascent in the trend interesting us is closely connected with the International Society of Soil Science. Pantanelli (1924, Conference Intern. Pedology, Rome) reported on the use of moulds for testing the phosphorus requirement of soils. He cultured *Aspergillus niger*, *A. Oryzae*, and *A. flavus*. Pantanelli found that *A. niger* and *A. Oryzae* gave the best results.

At the 1-st International Congress of Soil Science at Washington in 1927, the information of Winogradsky, reported by J. Ziemiecka and her demonstrations played a very essential part, as will be seen from further statements. The problem is already being studied on a world-wide scale in the most various countries.

The method of soil plates suggested by Winogradsky found an especially wide application. Besides the work of the authors of the method (Winogradsky et Ziemiecka, 1928) conducted in Paris, there appeared studies of Ziemiecka for Poland (1929) and England (1932). For France we have also the work of Guittonneau (1929), for the USSR the studies of Uspensky (1930) and Kriutchkova (1930), as well as of Issakova (1929), Simakova (1932), Minenkov (1932).

In America we must first of all note the systematical studies of Sackett and Stewart (1931). In Germany, the school of Niklas continues to develop its work (1930, 1932). The methods of Winogradsky are here specially propagated by Keller (1932). Benecke and Söding (1928) set up questions of a more general character. In Japan Itano and Arakawa have utilized it (1930).

The use of soil plates provided such great advantages, that other improvements of the methods with *Azotobacter* have been set aside into the background. Thus, for instance, the addition of albumen to the liquid culture and the use of mixed cultures (Truffaut et Bezsonoff) were not used by anybody else besides the authors themselves.

The method with *Aspergillus niger* also received further development. In Germany Niklas and collaborators (P-

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schenrieder, Trischler) joined it especially in relation to potassium (1930, 1932); Niklas, Poschenrieder and Frey (1931) used the same organism to test for available magnesium; serious attention was also given to this method by Benecke and Söding (1928) as well as by Lohmann (1930). In our Union Butkewitch returned to *Aspergillus* (1932), and experiments were also set up by Simakova and Bovshik (1932). In America Smith, Brown, Schlots (1932) reported the results of a comparative study of the Niklas' *A. niger* and Truog — chemical methods for available phosphorus. They found the two methods to check very well. They also concluded that the citric acid present in the medium is responsible for the solution of the soil phosphorus in the *A. niger* method, and the same results could probably be obtained with a simple citric acid extraction of the soil.

Recently by E. Br. Fred and collaborators in a study of the *A. niger* method for the determination of available potassium in soils, attention was given to the following points: a) adaptability of various strains of *A. niger*; b) maintenance of stock cultures; c) influence of reaction, various salts, acid radicals and minor soil constituents and stimulants; d) application of the method to the quantitative determination of available potassium by means of analysis of the mycelium for potassium, and by interpolation on a specially constructed curve; and e) comparison of results with other methods and field tests. It is concluded that the test is simple and reliable and may be used in a practical way for the determination of potash needs of soils.

Besides this, the use of new organisms — (*Cladosporium herbarum* (Benecke and Söding, 1928); yeast for phosphorus (Seidel, 1931); *Rhizopus arrhiza* for phosphorus, potassium, and calcium (Seidel, 1931) and especially of soil algae (Benecke and Söding) is planned.

In examining the new studies, we see that the successes and the failures of separate authors are closely connected with those soils on which they worked and with the peculiarities of fertilizers, which were not sufficiently accounted for.

Besides this, it is very important to note, that the problem of microbiological calculation itself greatly varies according to local conditions of nature and farming. Correspondingly, the methods must also change. With inalterable methods arise difficulties which cannot be overcome. And, on the contrary, a whole series of difficulties falls off, if we do not view the matter in a too general way.

It must also be remembered that our methods must find a fitting place in the system of other investigations. Just those authors succeeded in securing good results (Christensen, Niklas, Sackett and some others) who had solved this very question.

According to the above mentioned, I shall state the basic moments of the work according to the following scheme:

1. Questions of methods in connection with peculiarities of soils and fertilizers.

2. The question of experiment schemes in connection with problems and schemes of inspection.

3. The outlooks of microbiological estimation of soil in the general system of the development of soil science and agriculture.

We assume this scheme only in order to maintain a certain sequence in the review. We cannot abruptly border off the individual questions.

The questions of methods in connection with peculiarities of soils and fertilizers

The method of soil plates with *Azotobacter* being distinguished by a series of great advantages, creates at the same time also a series of essential difficulties, when the peculiarities of the soil are not accounted for. We see a decided failure in the case of Itano (Itano and Arakawa, 1930) in Japan on acid, strongly moistened soils of rice fields. With the addition of mannite the soil swells, and various fermentators depress the development of the *Azotobacter*. If we now turn to our soils, we shall find all the transitions from the ones Winogradsky had to cases analogous to those of Itano. Our more alkaline soils (in part chernozems, solonetz and solonchaks, gray-soils) provide a good growth of *Azotobacter* without any additions of cultures from without simply by adding mannite. The northern acid soils do not afford any growth of *Azotobacter* and swell even in the dish with an addition of lime and with additions of *Azotobacter* from without, whereas in the interval we find soils producing growth only with the addition of *Azotobacter* culture in the limed dish. In connection with acidification of the soil the appearance of mould is also very usual. The work of A. P. Kriutchkova (1930) has shown that bulging of soils may be obviated by providing drainage out of charcoal. In this last case the oxidation-reduction potential does not fall very low (with $rH \leq 24$ the growth of *Azotobacter* is quite possible), — the fermentations creating gases and acidulation are retarded.

We consider it necessary to introduce drainage even in such cases when no fermentation is perceptible, but it goes on, however, at a slow rate and acidifies the soil. If, in this last case, no drainage is provided, we get an exaggerated need of lime and an understated want of phosphorus. Issakova, Domracheva and Simakova, reckoning with our data on the acidification of soil tried to avoid the use of drainage, by decreasing the layer of soil.

According to our data for a series of soils, this is not sufficient. It is possible to ascertain this in each separate case by determinations of the decrease of pH.

However, the introduction of mannite in a series of soils not only leads to the decrease of rH and pH. Even in such cases, when we compensate the decrease of pH by lime, on some soils (some podzols from Torjok, government of Tver, podzols of Engelhard's Experimental Station, red soils of the Batum region and on many others) we do not obtain any growth of *Azotobacter* even in the case when it is added, or when the *Azotobacter* already existing in the soil does not manifest itself. An addition of 0.1 per cent of agar-agar or silica gel (see below p. 100 a, 109) to the water helps to obviate this difficulty. We seem to encounter in those cases a surplus dissolution of iron, which hinders the *Azotobacter*'s development, if no buffers are introduced in relation to the iron and if the degree of ionization of this metal is not decreased.

It is, certainly, impossible to deny in this question another side of the matter. *Z i e m i e ċ k a* (1929) ascertained that the addition of silica gel allows the cultivation of *Azotobacter* upon soils poor in phosphoric acid. According to *Z i e m i e ċ k a*, the *Azotobacter* behaves as the high plants in *L e m m e r m a n n*'s experiments. However, we had to deal with many cases, when the full rate of lime, potassium and phosphoric acid did not help: i. e. the *Azotobacter* did not grow—not on account of a deficiency in phosphoric acid. However, agar-agar and SiO_2 helped also in this case. Further, on many soils giving a good growth of *Azotobacter* without additions of agar-agar or SiO_2 the adding of gels decreases or completely excludes development. Therefore we consider, that in the first case the gels remove the harmful action of the excess of iron, and in the last case they lower the activity of the iron below the norm. We see therefrom, that it is necessary to work out the methods calculating the soils on a regional scale.

It is, certainly, not by accident that *W i n o g r a d s k y*, working in France, instead of setting up the two series of *C h r i s t e n s e n*'s experiments, only sets up one and does not make any additions of *Azotobacter* to the soil. Nevertheless, without any addition of *Azotobacter* we would have to give up the examination of precisely those soils which especially need lime. Besides, the addition of *Azotobacter* reduces the time of the experiment to 24 hours. Therefore, we (1930) (the same as *S a c k e t t* (1931) in America) always add a definite dose of standard *Azotobacter* culture on the northern soils.

The value of the additions of *Azotobacter* cultures when calculating phosphoric acid was noted also in the last work of *J. Z i e m i e ċ k a* (1932).

On northern podzols we assume the rate 2 000 000 cells of young (3-5 days) cultures of *Azotobacter chroococcum* or *Az.*

agile per 1 g. of soil. More to the south less can be added. Turkestan gray soils often provide an excellent growth of *Azotobacter* without any additions. But, depending from the statement of the question, it is often expedient to add *Azotobacter* also here, and it must be emphasized that the question of the dose of *Azotobacter* is in general closely connected with the way the question is formulated (see below).

Inasmuch, as experiments with *Aspergillus* are set up in liquid cultures, this method seems to be less connected with the type of soil. However, it is just this very circumstance which makes it simply inapplicable in many cases. Thus, the question of the latter drops off when acid soils are studied in connection with liming. For the methods with *Aspergillus* there also arise unsurmountable difficulties on alkaline soils, rich in phosphorus and lacking in mobility, in order to determine phosphorus. The inevitable acidification when using *Aspergillus* creates here such displacements in the solubility of phosphoric acid, that we completely wander away from field conditions.

Besides the afore mentioned difficulties there arise before microbiological methods a whole series of difficulties, which stand out especially abruptly in the relation of nitrogenous fertilizers and should be quite differently treated in relation to individual soils with their peculiar biology.

A considerable part of those difficulties has already been enumerated in the work of Benckke and Söding (1928) and may be summarized as follows:

1. All soil conditions change in a resolute manner, when we flood the soil samples with a liquid.
2. The activity of soil organisms changes or is completely excluded, if the soil samples are sterilized.
3. Those transformations of matter, which in a natural environment have a normal course during several months of the vegetational period, cannot in the same measure be manifested during experiments of short duration.
4. The temperature in the thermostate or vegetation cupboard also calls forth changes as compared with natural conditions.
5. Finally, only with the most cautious critical approach is the transfer to other plants of the results obtained from one or several objects admissible.

As regards the first point, it is not so very alarming. It is known that with higher plants water cultures did not succeed at the beginning. On the contrary, with improvement of nutrient solutions, it became possible to cultivate also the steppe-grasses in water culture and in conditions not especially aerobic. We believe that with lower organisms the difficulties arise not so much from the flooding with water, as from unsuitable nutrient solutions. Well, let everything depend from the «swamp» of water cultures. This is again not at all so alarming, for it is

possible to advance in the steps of S. Winogradsky and use the soil plates. If to the latter are added improvements, which are introduced in our laboratory by A. P. Kriutchkova, then the difficulties will completely fall off.

The second point is also not very alarming. In many of the most important cases it is possible to dispense with sterilization. Thus, if the question of phosphorus is being solved, it is best of all to use *Azotobacter*. This allows the exclusion of the influence of uncalled for nitrous transformations in an unsterile soil in the artificial environment of the experiment (see details below in the description of our method). If we are interested in potassium, we can weakly acidify the liquid and use *Aspergillus*, this will also set aside the need of sterilization. For problems with nitrogen we shall base ourselves on algae. This will exclude the influence of organic matter especially alarming in unsterile conditions.

It is much more difficult to cope with the difficulties indicated in the third paragraph.

We cannot evaluate all the possible transformations which the nitrous matters will undergo during summer. It is obvious, that by means of experiments of short duration we can answer, at the most, to the question about the supply of nitrogen being mobilized, supposing, that during the summer meteorological conditions will remain unchanged. But such a stating of the question has as yet not been realized. We assume that it can be realized. At any rate, it is clear that the principal difficulty of evaluating the nitrous supplies lies just in this domain. As this «domain» is essentially connected with microbiology, we assume that, notwithstanding all the difficulties, this paragraph is accessible to the microbiologist.

In truth, the difficulties arising from the fact that the content of nitrogen available during a certain period of time is not at all the one that can be obtained by the plant during the summer, — are difficulties which cannot be set aside by any chemical determination. Besides this, as the meteorological conditions change year in year out, the question is not solved even by one year of field experiments. If we take into account that in the conditions of our Union, we face the continually growing technique of soil tillage, it will become clear that the difficulty noted is not a difficulty specially of the microbiologist, — it is a difficulty connected with the fact that we wish to solve a concrete synthetic problem without any of the conditions which concretize it. In order to proceed from a separate test with determined conditions to a prognosis concerning the results of yield, we must know the curve of microbiological processes for the given type of soil under various meteorological conditions, with suitable cultures, and technical measures applied during that year. We have thus passed over to the question of the system of investigations, on which we shall dwell below.

It must be noted, that our chernozems with a vigorous productive horizon offer additional difficulties of a similar type. The roots of the high plant suck here their nourishment not only from the arable layer. Depending from seasonal conditions, their distribution in this case changes. In such cases, it is especially difficult to proceed from a small clod of soil to a layer of over 1 meter thick. But, we must again emphasize that in this case as well, all the chemical determinations and vegetational experiments are also of no avail. For shallow podzols this difficulty does not exist.

The following fourth paragraph of B e n e c k e and S ö d i n g must be approached from two sides: First of all we must intensify it. In microbiological experiments we often change not only the temperature alone. Very often we do not even remark that by the way we perform a whole series of changes. Thus, for instance, in adding mannite W i n o g r a d s k y did not pay any special attention to the fact that he was changing the acidity of the soil and the oxidation-reduction potential.

Whereas such correlations, certainly, disturb the natural conditions, and intensively respond to the reaction of the organism, which is used by us. However, recent years precisely brought something to help to disentangle those difficulties.

We must not now blame temperature for everything, but at the same time the uncalled for changes may be sufficiently fully determined by a comparatively small number of easily determined constants. Thus, if we do away with the cases of change in the basic nutritive substances examined above, before us will principally remain the changes of acidity and of the oxidation-reduction potential, which can be easily determined. At one time, the variations in the activity of heavy metals provided unsurmountable difficulties. However, the use of a special modification of the buffer principle (E. E. U s p e n s k y, 1924; E. F. H o p k i n s, 1930) and the introduction in addition of agar-agar, etc. solved this case as well.

The following, fifth paragraph, is also such a one wherein each year brings more and more clearness. When we did not at all know the individual physiology of cells and organisms, it seemed that diversity is unsurmountably great. In connection therewith the question of the transfer of results from one organism to another seemed infinitely difficult. But, at present, the organisms align in series, and corresponding transfers are no more alarming.

Besides this, the presence of peculiarities in definite organisms does not make them quite peculiar. On the contrary, we often come across an astonishing uniformity. For instance, the work being at present carried out by M i t s c h e r l i c h is in many cases the transfer onto higher plants of the laws established long ago by R a u l i n (1869) with mould fungi. Thus, it must

be noted, that Mitscherlich's unit of nutritive substances is essentially the same thing, as Raulin's coefficients. According to Raulin the reaction of efficiency of P_2O_5 and N lies between 4 and 5, i.e. within the same range, as Mitscherlich has it. For K. Raulin emphasizes the variation of coefficient depending from the presence of Na and other salts, i.e. we again have the correlation of Mitscherlich.

Besides the most essential and general difficulties analysed above, Benecke and Söding dwell when describing the individual experiments on some of the more individual difficulties; one of such remarks of Benecke and Söding has, I dare say, a more general importance. Benecke and Söding reckon with the possibility, that the soil being studied contains some kind of poisonous or stimulating substances. In order to set aside this difficulty the authors themselves point out an excellent issue. They add a series of retorts, which are provided besides the complete nutritive mixture, also with an addition of the soil being studied. In the case of the presence of poisonous substances in the soil a decrease of yield should be observed as compared with the complete nutritive mixture, but without the soil. On the contrary, in the case of the presence of stimulants, under the same conditions, an increase of yield should be observed.

Thus, we see that all the difficulties, which called forth pessimistic reflections from Benecke and Söding, may be set aside in some way or other or, at any rate, may be removed to a certain limit.

The questions of experimental schemes in connection with problems

1. General considerations

It is quite comprehensible that the majority of investigators strived to confront the results of microbiological estimation with field experiments. Without any possibility of establishing suitable correlations the modes of procedure lose their agronomic value. However, if we examine the separate studies with more attention, we shall see that different investigators make their juxtapositions diversely, and call for microbiological methods in order to solve essentially differing problems in the general system of agronomic examination or control. By this are largely conditioned the differences in the investigation schemes and in the details of modes of procedure.

Microorganisms represent a very peculiar reagent, which is not expended, but which, on the contrary, grows while producing reaction. Therefore, depending from the initial dose of microorganisms and from the duration of the experiment, we answer quite different questions. For the agronomist of a special interest is such a combination when we have a large dose of microorganisms

and perform the count comparatively rapidly. In this case it is possible to assort such correlations, that the present assets of the investigated factor in the soil solution is rapidly exhausted by the culture, and the calculated increment appears in its essence to depend only from the rate of mobilization of the easily available supplies of the factor being investigated. In this case we provide an answer about the present available supply, which is, of course, especially important for the yield. Differing from purely chemical determinations, — we establish thereby not the level, so to say, of soluble phosphates, but the availability of the basic supplies. We determine not the instant activity of the factor, but its activity with a determined force of suction. Obviously, this is not the general amount of the given factor and not its soluble part, but just what is needed by the plant. The whole secret consists only in the selection of such an organism which would be such a given factor in conformance with some cultural plant.

Besides this, there must be a sufficient amount of the organism's cells, i. e. additions of culture are necessary. It is quite comprehensible that such an approach is only possible for methods with *Azotobacter* in soil plates, and is impossible for usual methods with *Aspergillus niger*. For our podzols per 1 g. of soil we have to take with such a fixing 2 000 000 cells of *Azotobacter chroococcum* or better of *Az. agile* — in the case of phosphorus determination, and 1 000 000 cells in the case of potassium determination.

Another essential advantage of biological tests, as compared with chemical ones, is used by us only in such cases, when we take an organism peculiar to cultural soil. With such an approach we do not enclose ourselves into narrowly estimating methods, and we are able to notice something novel, resting in quite another plane. Thus, if we work with *Azotobacter* and the experiments do not succeed even with additions of Ca, P and K, this is not at all so bad. We notice in this way some novel relation. We were able thus (see above, page 97) to observe the magnitude of the action of the surplus of iron. S. I. K u s n e t z o w (see this volume pag. 113—131) interpreted in such a manner the question of the poor effect of nitrous fertilizers in northern Turkestan. At present, along this course goes on the study of various phases of the action of phosphates on different types of our soils. In order to solve such problems, it becomes necessary in separate cases to pay attention also to the instant state on the present day of one or the other ingredient in the soil. Here we have not to make any large additions of *Azotobacter*, and we can follow it not by the development of colonies, but by its propagation in the soil, etc. In this way, those variants of methods imperceptibly develop into C o n n's modes of procedure (1930) with *Bac. globiforme*.

It is evident that such an approach does not enter into the system of examination, but the system of observations and of the scientific daily management of the field. It is also obvious, that here we can rest on *Azotobacter* and many other organisms, but not on *Aspergillus niger* which is quite foreign to good soil.

It must also be noted that the methods with *Azotobacter*, differing in this from chemical modes of procedure, account for the action of some factor not in an isolated manner, but in the environment into which it gets in the given concrete case. The shape of the factor into which the *Azotobacter* is introduced, is also accounted for in this case. Thus, upon gray forest soils carbonate lime does not act either upon the *Azotobacter*, nor upon the field cultures. Slacked (caustic) lime, on the contrary, acts upon the *Azotobacter* and the field cultures. Such correlations facilitate immensely the analysis and render microbiological methodics accessible even for such cases, which demand a complicated chemical analysis.

All the above mentioned renders the microbiological methods indispensable in the scientific management of current work of a separate agricultural unit or district. But it is also important in the examinations of the type and opportunities of a soil. In such cases, certainly, a variant with additions of important doses of microorganisms has to be applied. Those investigations are the more easily adjusted with the field data, the less the tillage and the cultivation of the field are changed. In our conditions of very abrupt shifts in the trend of the rise in farming methods, even a simple evaluation does not provide any answer as to what will really occur in a couple of years. It is, therefore, necessary to supplement the narrowly evaluatory methods of Mitscherlich's kind, by methods of diagnostics, which deepen our conception of one or the other effect. As we have already noted, the methods with *Azotobacter* or with other typical not very compliant soil organisms will also provide something in that direction.

It is also known long ago that the microbiological methods can be extended to the study of the availability of phosphoric acid in various phosphates [Niklas, H., Scharrer, K., and Strobel (1925); Truffaut and Bezsonoff (1927), and others] and also to the study of the importance of admixtures to phosphates (Truffaut and Bezsonoff, 1927).

The examinations which we have to carry out are of various types. We have accordingly developed several schemes. Scheme I is comparatively closer related to the original one proposed by Winogradsky. It has been extensively verified by us on most varied soils, and does not encounter any difficulties where there are some field experiments for comparison.

Scheme II in itself already provides a more precise answer. It may be, therefore, used, on the one hand, where nearly no field experiments on such soils exist. On the other hand, it is suitable as a substitute for minute fractional field calculation. It must be noted that in relation to phosphorus we usually observe a simple proportionality in the action of additions made in the field and in the experimental plates. This rendered nearly superfluous the III-rd scheme which was to serve for the calculation of the total supply of phosphorus in the soil.

Let us imagine that we have one of the most difficult cases: we must solve the question of liming some new soil hitherto uncultured, and that there are no experiments with analogous soils, in the neighbourhood. For this we use the following scheme.

To the soil investigated, and to soils serving for comparison, into the separate portions are added, besides common mannite and *Azotobacter*, also various doses of chalk: 0; 0.125; 0.25; 0.5; 1; 2; 4 g. per 100 g. of soil. Let us assume that for comparison we take 3 of our loams from the Tiurikov field of the Scientific Institute on Fertilizers (Moscow), differing in the degree of podzolisation (weak, medium, strong). Then we get the following scheme which in our work is called II.

The numbers in the Table show the colonies of *Azotobacter* pro total surface of plate.

Additions in the soil plates	Degree of podzolisation			
	A: weak	B: medium	C: strong	X: studied
Mannite + 4% CaCO_3	2 500	350	4 500	2 760
» + 2%	2 960	1 780	1 760	2 060
» + 1%	3 020	1 680	1 680	2 050
» + 0.5%	3 040	1 060	920	1 600
» + 0.25%	2 600	1 040	720	1 200
» + 0.125% CaCO_3	1 420	73	0	400
Conclusion: the soil studied (x) lies between A and B.				

From this, the most complicated case, when a whole series of plates has to be set up, we shall pass over to the more simple one, when on the soil of a corresponding type there exist, in the given district, field experiments with doses of lime. Thus, for instance, for the Dolgoprudnoye Experiment Station of the Institute on Fertilizers, we have standard fields which received the following doses of lime: 0; 2.25; 4.5; 9; 13.5; 18; 22.5 ton/ha.

On the basis of field experiments we assume the rate of a dose—10 ton/ha.

Taking the soil studied, we set up with it, according to the so-called I scheme, the following dishes: I—with mannite;

II — mannite+Ca+P+K; III — mannite+Ca+P; IV — mannite+Ca+K; V — mannite+P+K.

Let us assume that we obtain numbers of colonies corresponding to the standard, which has received in the field 2.25 ton/ha. Therefore our investigated soil differs from the natural soil of the DEST., but corresponds to the latter with an addition of lime of 22.5 ton/ha. If, for the soil of DEST. we have fixed a dose of 10 ton/ha of lime, then our investigated soil needs a dose of 10 ton/ha — 2.25 ton/ha = 7.75 ton/ha.

It is obvious that in the majority of regions we shall come across intermediate cases between our II and I schemes. In those cases we will have to combine the II and I scheme. We shall have to set up the experiments according to scheme I, but with several doses of additions of lime into the plates, i. e. applying partly, also, the II scheme. However, as the existing experiments will provide a certain approach to the degree of podzolisation of the soil, we shall not be obliged to take 3 standards; generally it will be possible to reduce them to 2 and also to diminish somewhat the number of doses.

Thus, for instance, if the soil lies in the region of medium-weak podzolisation, we shall only take doses below 1 per cent; if, however, the soils are from a medium-strongly podzolised kind, we shall take doses only of 1, 2 and 4 per cents.

As regards phosphorus, in the presence of a great number of experiments for various regions, the choice of a standard for scheme II becomes considerably easier, and at the same time, the answers based on the latter are sufficiently accurate.

Therefore, the scheme I may be used in a series of cases in its simplest qualitative-quantitative modification without any comparison with the field only in order to mark the type of soil. While the more accurate definition may be performed by scheme II.

When there is no field experimental material, in a completely new region, the use of scheme II on phosphorus gives a perfectly accurate determination of the scale of local soils. However, inasmuch as for the standard in this case is taken dry soil from another region, a certain error is connected with the change in the state of phosphoric acid during desiccation. But, inasmuch as those variations are not so much related to the mobilized phosphoric acid, as to the soluble one, and that, besides, it is possible to choose a soil either changing negligibly or already well studied, this error may be nearly liquidated.

2. Details of *Azotobacter* methods of the Institute on Fertilizers

I Scheme

The scheme I is used for orientative analyses of soils and gives an answer on the provision of the latter in available phosphorus, potassium and calcium.

This scheme includes the following series — soil plates: I—0; II—CaPK; III — CaP; IV — CaK; V — PK.

Mannite and Azotobacter cultures are introduced into all plates (see below).

In this scheme phosphorus is introduced in the form of a mixture of Na_2HPO_4 and $+\text{NaH}_2\text{PO}_4$ with pH — 7.0 — 7.1, calculating 10 mg. of P_2O_5 per 100 g. of soil.

For this, when preparing the soil plates (see below), a solution containing 5 mg. of P_2O_5 per 1 cc. is taken, and 1 cc. is introduced into a plate with 50 g. of soil (a solution per 100 cc. contains 1.608 g. of Na_2HPO_4 , $12\text{H}_2\text{O}$ and 0.320 g. $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$).

Potassium is introduced in the form of K_2SO_4 reckoning 2.5 mg. of K_2O per 100 g. of soil. For a plate with 50 g. of soil 0.5 cc. are taken, containing 0.462 g. of K_2SO_4 per 100 g. CaCO_3 is added at the rate of 1 per cent of the weight of soil.

As will be seen in the description of the preparation of soil plates, the moisture in all the plates must be equal, therefore in the I-st scheme, when adding water, the amount of liquid introduced with the reagents is discounted or the moisture is previously levelled. In order to equalize the moisture, into plate I is introduced 2 cc. of water, into the second one — 0.5 cc. (for wetting the calcium), into the third 0.5 cc., into the fourth — 0.5 cc.

The inference about the soil is given on the basis of the number of colonies having grown up on the surface of the soil plates.

If the number of colonies on all the plates is equal, this shows that the soil is well provided with P_2O_5 , K_2O and needs no lime.

If the second plate shows an increment as compared with the III-rd, then the soil is responsive to potassium. The same may be said in relation to phosphorus and lime, when comparing the II-d plate with the IV-th and V-th.

II. Scheme for phosphorus

In fixing the experiments according to this scheme, P_2O_5 is added to the soil in various doses.

To the soil is also added mannite and the culture of Azotobacter, as in the first scheme and for a background — K_2O and CaCO_3 .

K_2O is added in the form of K_2SO_4 reckoning 2.5 mg. per 100 g. of soil.

CaO is added in different quantities depending on the properties of the soil. On strongly podzolized soil, 2 per cent; on medium and weakly podzolized ones, 1—0.5 per cent.

On chernozems, where the growth of Azotobacter is possible without any lime, CaCO_3 is not added. On leached and degraded chernozems lime is added in small amounts of from 0.25 per cent to 0.5 per cent. Into soils prepared in such a way are further introduced increasing doses of P_2O_5 . The I-st plate is left as a control, and into the second one phosphorus is introduced, reckoning 1.5 mg. per 100 g. of soil; into the III-d, 3 mg.; into the IV-th, 45 mg.; into the V-th, 9 mg., and into the VI-th 18 mg. of P_2O_5 .

The doses assumed for this scheme are taken from a calculation for field doses: 45 kg./ha, 80 kg./ha, 135 kg./ha, 80 kg./ha, 135 kg./ha, 270 kg./ha and 540 kg./ha of P_2O_5 is introduced in the form of a mixture of Na_2HPO_4 and NaH_2PO_4 .

In order to procure such a solution, per 100 cc. of distilled water is taken — 2.894 g. of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 0.579 g. of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. This solution included 9 mg. of P_2O_5 per 1 cc., and therefrom 1 cc. per 50 g. is introduced into the VI-th plate, where a dose of 18 mg. of P_2O_5 is given per 100 g. of soil. For the following doses of 9, 4.5, 3, 1.5 mg. per 100 g. of soil, the solution is diluted 2, 4, 6 and 12 times respectively; from the diluted solutions 1 cc. is also taken per dish.

The Azotobacter possesses an exceptional sensitiveness to phosphorus, and the number of colonies strongly fluctuates depending from the presence of assimilable phosphorus in the soil. The increase in the number of colonies from the introduction of phosphorus may go on to the highest dose of

P_2O_5 , or else only to a certain limit after which the further additions do not call forth any increment or else depress the Azotobacter's development.

As a result of the count of the colonies on the soil plates, it is possible to characterize the soils in the following way:

I. The soil contains much phosphorus accessible for the plant, if the introduction of phosphorus additionally does not show on the growth of the Azotobacter and if large doses oppress.

II. The soil reacts weakly to phosphorus, when small doses of P_2O_5 have an action, and further increments do not provide any increment in the number of colonies.

III. Medium soils need phosphorus, if on small doses the increment is negligible, but the following doses of 3—4.5 mg. give the same growth, as on large doses.

IV. With a strong need of the soil in phosphorus there goes on a gradual addition from an increase of P_2O_5 up to the maximum dose.

Comparison with standard soil defines more accurately the answer within each of the 4 categories enumerated above.

II. Scheme for lime

This scheme is used when there exists only the question of liming, and it is necessary to estimate more accurately the degree of podzolisation and the need of the soil in lime.

It differs from scheme I by the fact that the soil receives various additions of $CaCO_3$. This scheme consists in the following. Seven portions of soil each of 50 g. are taken. Into all of them is added: mannite, culture of Azotobacter, phosphorus in the form of a phosphate mixture computing 5 mg. of P_2O_5 per 100 g. of soil, potassium in the shape of K_2SO_4 calculated 2.5 mg. of K_2O per 100 g. of soil, various doses of $CaCO_3$. The first dish remains as a control, into the II-d is introduced — 0.125 per cent, into the III-d — 0.25 per cent, into the IV-th — 0.5 per cent, into the V-th — 1.0 per cent, into the VI-th — 2.0 per cent, and into the VII-th — 4.0 per cent of $CaCO_3$.

Soils of a strong degree of podzolisation provide a great number colonies, beginning from 2 per cent and higher. On medium-podzolised soils Azotobacter grows well with doses of 0.5—1 per cent. Weakly podzolised soils provide a considerable number of colonies with the smallest doses (where on soils of a medium and strong degree of podzolisation the Azotobacter does not yet grow). On the contrary, with 4 per cent and 2 per cent — the weakly podzolised soils give less colonies, than with 1 per cent. Thus, it is possible to determine the degree of podzolisation of one or the other soil and of its need in liming by the number of colonies on the soil plates with various dose of lime. In this case a comparison with the respective experiment of a standard soil defines more accurately the answer also in the case of lime.

II. Scheme for potassium

According to this scheme, various doses of K_2O are introduced. To seven (in some cases less) portions of soil are added mannite and Azotobacter culture. For a background 1 per cent of $CaCO_3$ (in the case of strongly podzolised soils 2 per cent of $CaCO_3$ is added; to carbonate soils $CaCO_3$ is not added) and phosphorus in the shape of a phosphate mixture of 5 mg. of phos. per 100 g. of soil.

One plate is left as a control, to the others are added various doses of K_2O : 0.01—0.005—0.0025—0.00125—0.000625 and 0.000312 per cent. For this, per 50 g. of soil is introduced 1 cc. of solution containing in every two cubes: 10; 5; 2.5; 1.25; 0.625 and 0.312 mg. of K_2O .

In the case, when the soil is well provided with potassium, the dishes with different doses of K_2O do not differ in number of colonies.

With a growth in the doses of potassium occurs a gradual increase of the number of colonies up to a determined maximum, after that there comes a reduction. If the soil includes accessible potassium, but its amount is limited and the soil responds weakly to the addition of potassium — the optimal

development of the *Azotobacter* will on small doses of K_2O , and its further increase will not produce any increment. Thus, even when examining and still more, in counting the colonies it may be seen what class the investigated soils should be referred to. As an example the following soils may be mentioned.

Doses of K_2O in per cent	Soil № 1	Soil № 2	Soil № 3
0	5 880	1 800	2 410
0 00031	5 720	4 730	2 760
0 000625	5 800	5 750	2 680
0 00125	5 730	6 540	2 640
0 0025	5 930	6 390	2 540
0 005	5 780	5 640	2 540
0 01	5 710	5 770	—

Notice: In work according to this method, the solving of the salts has its importance, especially the solving of sodium salts by potassium.

Besides Na-phosphate, secondary phosphate of calcium may be used, which is also well assimilated by the *Azotobacter*.

The fixing of the experiment somewhat changes in this case.

The soil, mixed with mannite and $CaCO_3$, is shifted to one side of the plate and the plates are placed slantingly. $CaHPO_4$, weighed out in portions of 20–30 g., is filled into the plates 4 cc. of *Azotobacter* culture and 2 cc. of distilled water per plate are added, for a better distribution of the $CaHPO_4$. Further is added the necessary amount of water, and the whole is mixed with the soil to a consistency of paste.

Preparation of soil plates

Depending from the scheme, one or another number of portions from the prepared soil of 50 g. each, are transferred into cristallisators of a 10 cm. diameter. To each portion is added 0.25 g. of mannite and *Azotobacter* culture at the rate of two million per 4 g. of soil (preparation see below). Besides this, are also introduced the salts foreseen by the scheme of the experiment. First of all into the plates are added the dry reagents, and they are then mixed with the soil by means of a spatula, then salts in the shape of solutions and the weighed cultures of *Azotobacter* and water are poured in.

Adding water

Into all the plates the water is added in equal amounts; for this is used a 10 cc. pipette with divisions up to 1/10.

Into the first plate the water is poured gradually, so as not to pour more than needed until the soil is transformed into an easily smearing, but not liquid mass. In order to secure the necessary consistency, to the podzolised soils water is added: 80–85 per cent of the total moisture capacity, and to chernozems: 90–95 per cent (for some soils variations are possible).

For a better distribution of the fertilizers, the soil is carefully mixed up with a spatula.

Filling of the plates and draining

After mixing up, the content of the plate is divided into halves with a spatula and each part is filled into a small Petri cup of 5 cm. in diameter and 1 cm. high.

In the Petri dishes drainage is provided out of cut up charcoal moistened to its full moisture capacity. In order to extract the possible soluble compounds, the charcoal is left in the distilled water over night, or it is boiled for some time.

Before use the charcoal is transferred into a funnel, then is rinsed out with water and after this, when all of the water has run off, the charcoal is put into the plates approximately filling half of each plate.

A glass tube somewhat protruding over the rims of the plate, is fixed for communication with the air.

The soil is then stirred with a large spatula into the charcoal and is then smoothly levelled with a small spatula. In each plate beneath the lid is placed a small round of filter paper in order that the water evaporating and precipitated on the lid should not get into the surface of the soil.

Addition of Kaolin

If the soil is sandy, kaolin is added for greater binding. The kaolin is previously rinsed in the following way: pure hydrochloric acid is poured over it for 2-3 days, several times during that period it is shaken and, finally, rinsed with distilled water until a complete disappearance of chlorine.

Addition of silica gel and agar-agar

A 0.1 per cent solution of agar-agar is added to the soil instead of water and mixed up.

The silica gel is added, calculating 1 per cent of SiO_2 for all soils.

Preparation and addition of the Azotobacter suspension

The culture of Azotobacter is introduced into all soils in an equal amount, viz. 2 mill. cells of Azotobacter per 1 g. of soil (in the case of experiments on potassium, 1 million per 1 g. of soil is introduced).

A 3-5 days culture grown on slanting agar is washed off with distilled water into a small flask with a ground stopper. In order to better separate the cells, the suspension is strongly shaken during 5-10 minutes until small clods of slime become apparent. After this a drop of this suspension is deposited into the glass of the counting chamber, it is then covered with a coverglass and is examined under microscope. If the distribution of the cells is sufficiently uniform and the number of cells does not exceed 25-30 per one square, the count is performed.

It is recommended for comparison to use a comparer employed for calorimetric determination of pH, and to compare on a blue background; for this water tinted with methylene-blue or other dyes, is poured into two of the rear test-tubes.

Culture medium for Azotobacter

We use Ashby's medium: mannite 15 g., phosphate hydrogen potassium alkaline) 0.2 g., sulfate magnesium 0.2 g., chloride sodium 0.2 g., sulfate calcium 0.1 g., agar-agar 15-20 g. or the medium of Beijerinck.

The cultivation and counting of cultures

The prepared soil plates are placed into a moist chamber and are left in the thermostat at 30-32° C for 24 hours. After expiration of 20 hours on the surface of the soil plates, generally, grow up colonies of Azotobacter. After 24 hours, the colonies reach a marked size, but do not yet merge, therefore after 24 hours they are counted.

The count of Azotobacter is performed by means of a Neubauer counting chamber.

Conclusion. The importance of microbiological methods in the general system of agronomical soil investigations

As we see, by its simplicity, rapidity (within 24 hours), by the small amount of soils and other features, the microbiological mode of procedure can compete with the simplest chemical methods.

At the same time this method can abridge its «biologicity» (see p. 102) and not allow the evaluating determinations to become narrowly evaluative. Our method can serve as a key for opening up new questions, new approaches and paths in order to master the management of the soil's life. In this lies its special importance. We should not tear ourselves away from physiology and take a fancy to simplification.

All this is possible if we change the methods according to the type of soil, to crops, to the problems of separate agricultural units and districts, or to the plan of investigation.

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MICROBIOLOGICAL STUDIES IN THE IRRIGATED REGIONS OF TRANSCAUCASIA AND TURKESTAN

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I. Introduction

A series of phenomena observed when applying fertilizers to cotton fields in Turkestan and Transcaucasia, led us to observe the peculiarities of soil biodynamics in those regions. The Cauchi Experimental Station and other stations have long been recording the extremely rapid disappearance of large amounts of nitrates during irrigation (in a period of 7 to 10 days as high as 200 mg. per kg. of NO_3 was reduced to nearly 0), a very rapid decomposition of organic substances containing nitrogen (e. g. cottonseed cake), the low efficiency in many localities of nitrogenous fertilizers, particularly of ammonium sulfate, notwithstanding the alkalinity of the soil and its evident deficiency in nitrogen.

All these and other closely related problems were taken up at the annual agricultural conferences of the Scientific Institute on Fertilizers in 1925, 1926 and 1927. This Agricultural Conference adopted a special programme for the microbiological study of problems at the Cauchi Experimental Station.

It might seem that biodynamics of nitrogenous substances could be particularly well studied in Turkestan since, with a small total content of soil nitrogen (about 1 g. per 1 kg.), the gray soils (serozems) are distinguished by an enormous range of variability in the content of nitrates (up to 200 mg.), i. e., the annual dynamics of nitrous compounds is not masked here by a large fixed fund. However, studies carried out by local investigators in 1928 and 1929 did not furnish any definite answer to basic questions. Besides, the local workers discovered a series of new problems to some extent throwing light on new aspects of the matter, while, in other respects, they found a somewhat different interpretation when checking our experiments in other regions. Thus, the dependence of nitrate dynamics on rates of irrigation was ascertained (Ivanov, 1929, Gandzha). The Ak-Kavak

Station proved that the deleterious effect does not occur when irrigation takes place by infiltration. Furthermore, Cononova, emphasizing the fact that the Azotobacter can be isolated on silicagel only from irrigated soils, assumed that there is none on dry-farming tracts, whereas we know, on the one hand, that often the Azotobacter cannot be isolated on silicagel, though present in the soil, and, on the other hand, we had before us records showing that under conditions of greater droughtiness on salty and especially on loess soils, the Azotobacter must be looked for at considerable depths (Richter, Sabinin, Henkel and Zakharova). This, naturally, necessitated a checking of the data furnished by M. M. Cononova and others.

Finally D. A. Sabinin, who has done much work in Turkestan, brought forward a very essential observation during the discussion of a paper by Ziemiecka on the microbiological evaluation of soil requirements in fertilizers at the 2-nd Congress of Soil Science. He pointed out that Turkestan soils generally contain much Azotobacter and are, at the same time, in great need of phosphate fertilization, i. e., the Azotobacter is satisfied with other rates of phosphorus and, therefore, it is of no use for the solution of argicultural problems. D. A. Sabinin's observations did not call forth any definite objections on the part of the reporter.

The following programme of research seemed necessary:

1. To what extent is the disappearance of nitrates during irrigation connected with losses by denitrification, and with their biological absorption and transfer?
2. How actively do the other transformation processes of nitrogenous compounds take place?
3. To what extent is it possible to control denitrification during irrigation by regulating the irrigation and by using anti-septics?
4. Are there really no Azotobacter on tracts cultivated by dry-farming methods?
5. Can the usual microbiological evaluation of the need of the soil in fertilizers be applied to gray soils and what is the cause of energetic reaction to phosphates, and the weak action of nitrous fertilizers?

II. Losses and transfers of nitrates during irrigation

It is known that the main cause of the decrease of the amounts of nitrates during irrigation or other methods of increasing the moisture in the soil is generally attributed to the washing out of the nitrates.

The investigations of the Microbiological Subsection of the Scientific Institute on Fertilizers had shown that in such cases it was impossible to ignore the process of denitrification. Thus,

from T. M. Zakhárova's (1929) studies, it is evident that the autumn decrease in the amount of nitrates coincides with an increase in the amount of denitrificators. E. V. Litvinova, continuing the work of T. M. Zakhárova, proved that if the autumn moistening of the earth is accelerated by irrigation with distilled water, denitrification and a reduction in the amount of nitrates are still further increased. On the contrary, if the soil is protected from the autumn rains, there is neither an increase of denitrificators, nor a loss of nitrates. According to E. E. Uspensky's plan, the irrigated regions of Transcaucasia and Turkestan, to begin with, were to be observed still further in order to obtain still more definite answers to the question of the relation between the losses of nitrates by washing out and by denitrification. In these places, when the soil was irrigated, the water soaked only to a limited depth. Therefore the nitrates washed out of the upper horizons could only be transferred to the lower limit of the water penetration. If there is no denitrification, the total balance among all the moisture horizons must remain unaltered. Similar experiments were made in Transcaucasia at the Gandzha Experimental Station with the active participation of L. Rosenberg, assistant at the Scientific Institute on Fertilizers.

Table 1

Norm of irrigation 2000 m³. per ha. (Gandzha)

Date	Horizon	Total amount of bacteria in millions	Denitrificators of group <i>Bac. S. n. zeri</i> in millions per 1 g. of soil	Moisture in per cent	NO ₃ -nitrogen in mg. per 1 kg. of soil	Total nitrogen in per cent
10/VII 1930	0—10	835	0.5	6.2	21.20	0.156
	10—18	802	0.5	—	4.28	0.135
	30—40	1105	0.7	15.1	3.25	0.100
	60—70	610	0.2	10.2	1.22	0.103
	120—130	791	0.08	11.3	1.46	—
14/VII	0—10	—	3.0	23.9	3.01	0.147
	10—18	—	1.0	21.3	2.47	0.150
	30—40	—	1.0	21.7	1.90	0.082
	60—70	—	0.4	22.9	1.50	—
	120—130	—	0.08	12.2	traces	—
17/VII	0—10	—	6.0	20.9	—	—
	10—18	—	2.0	20.6	—	—
	30—40	—	0.7	20.7	—	—
	60—70	—	0.3	20.3	—	—
	120—130	—	—	19.2	—	—
4/VIII	0—10	—	0.6	18.0	39.63	—
	10—18	—	0.6	19.7	7.17	—
	30—40	—	0.07	19.8	5.02	—
	60—70	—	0.08	21.6	4.30	—
	90—100	—	0.02	—	2.32	—
	120—130	—	—	15.5	—	—

On a tract of land, where the norm of irrigation was 2000 m³. per ha., we observed a typical instance of the decrease of nitrates with a corresponding increase in the amount of denitrificators. From an examination of Table 1 we see clearly that no washing out of the nitrates into the deeper layers of soil could have taken place here. In fact, if we compare the analysis on the 10/VII, i. e. before irrigation, with the analysis on the 14/VII, the day following irrigation, then, according to moisture data, we can see that during 24 hours the moisture penetrated to a depth of about 100 cm. At 70 cm. the moisture of the soil had increased from 10.2% to 22.9%, while at a depth of 120 cm. the moisture remained unaltered, 11.3% — 12.2%. Thus, if the decrease in the amount of nitrates in the upper horizons had depended upon washing out, we should have discovered an increase in the amount of nitrates in the deeper horizons; but actually the analysis indicates a uniform decrease of nitrates throughout all the lower horizons to which the irrigation moisture has penetrated.

If we determine the average content of nitrates in a layer of soil 70 cm. thick in which all of the irrigation water is retained, both before and after irrigation, we find that the amount of NO₃ nitrogen has decreased on the average from 7.5 mg. to 2.2 mg. per kg. of soil.

Thus, the theory of the washing out of nitrates is eliminated, and the disappearance of the nitrates may be due either to the activity of denitrificators or to absorption by soil bacteria.

An analysis of Table 1 shows clearly that during an increase of soil moisture up to 12%—23% the amount of denitrificators in the soil sharply increases, and this certainly affects the decomposition of the soil nitrates. The decrease of nitrous nitrogen may also take place partially by means of biological absorption by transfer to proteinic nitrogen of bacterial bodies. In the given case it was impossible to establish this last process with certainty, in view of the possible errors in the method of calculating the total nitrogen content of the soil. However, it may be assumed that this process exists, since the total amount of bacteria increases after irrigation, and according to Kostytchev's data (1927), the increment of bacteria, amounting to 100 000 000 per g., involves an increase in the total amount of nitrogen of 17 mg., to each kg. of soil.

Comparing further the data of Table 2 with those of Table 1, we observe, essential differences together with some common features. As a matter of fact, when irrigating with 500 m³. of water per ha, the amount of denitrificators also rises. Thus, on the day following irrigation, when the moisture in the surface horizon of the soil had increased from 3.7% to 22.8%, the amount of denitrificators increased approximately 10-fold — from 800 000 to 7 000 000 — 5 000 000 per 1 g. of soil. But simulta-

neously the quantity of nitrates increased from 6 mg. to 14—17 mg. per 1 ha. of soil.

Norm of irrigation 500 m³. per ha (Gandzha).

Table 2

Date	Horizon	Total amount of bacteria	Denitrifiers group of <i>Bac. Stutzeri</i> in millions per 1 g. of soil	Moisture in per cent	NO ₃ -nitrogen in mg. per 1 kg. of soil	Total nitrogen in per cent
10/VII 1930	0—10	1 690	0.8	3.7	5.93	0.147
	10—18	281	0.8	—	2.30	0.142
	30—40	225	0.2	15.2	0.93	0.078
	60—70	112	0.7	—	traces	0.153
17/VII	0—10	1 747	7.0	22.8	14.4	0.211
	10—18	1 290	5.0	22.7	17.0	0.173
	30—40	1 347	—	16.4	traces	0.150
	60—70	399	0.3	—	traces	0.136
4/VIII	0—10	915	0.8	15.4	16.78	—
	10—18	1 227	0.6	16.9	14.15	—
	30—40	837	0.3	16.6	1.59	—
	60—70	742	0.3	11.6	0.92	—

It is obvious that denitrification also takes place when irrigating with 500 m³. of water per 1 ha. But, at the same time, not only nitrification increases but also nitrogen assimilation. In result we have a positive balance not only as regards the nitrates but also of the total amount of nitrogen.

When irrigating by means of infiltration, even with a norm as high as 2 000 m³. per ha., a positive balance of nitrates is observed; i. e. we obtain the same result as during irrigation by submersion with a norm of 500 m³. per ha.

On the basis of a determination of the total nitrogen and of the oxidizing potential of the soil, M. M. Cononova states that, contrary to the effect of irrigation by submersion, during irrigation by infiltration the processes of denitrification are absent.

Data of the Agro-Chemical Section of the Transcaucasian Scient. Research Institute for Cottonplant culture (Dashkevski in 1932) show that during irrigation by infiltration a partial drawing up of nitrates from the furrow to the ridge is observed.

Since the amount of nitrates in the soil is a resultant of a whole series of microbiological processes, the question arises as to how the denitrifiers behave in the furrow and in the ridge when irrigated by infiltration: do any processes of denitrification take place here, or is this simply the result of the migration of nitrates? Therefore, in experimenting with the introduction of phosphorous and nitric fertilizers, where particularly large amounts of nitrates were observed when irrigating, in the proportion

of 1000 m³. per 1 ha., samples were taken from the furrow and the ridge, and microbiological and chemical analyses were made.

Table 3

Irrigation by infiltration. Furrow (Gandzha).

Date	Horizon	Denitrifica- tors of group <i>Bac. Stazeri</i> in millions per 1 g. of soil	Moisture in per cent	NO ₃ -nitro- gen in mg. per 1 kg. of soil
15/VIII 1932 (before irrigation)	0-5	0.3	5.4	304.5
	5-25	3.0	5.7	42.7
	25-40	2.0	8.7	7.2
19/VIII (on 16/VIII irrigated 1000 m ³ . per ha.)	0-5	0.6	21.0	17.8
	5-25	2.0	18.4	3.5
	25-40	0.4	17.7	12.8
21/VIII	0-5	5.0	17.3	29.8
	5-25	0.6	17.7	13.8
	25-40	7.0	17.7	4.5
27/VIII	0-5	2.0	8.1	43.6
	5-25	2.0	10.3	10.2
	25-40	3.0	13.0	3.4

Table 4

Irrigation by infiltration. Ridge of the furrow (Gandzha).

Date of analysis	Horizon	Denitrificators of group <i>Bac.</i> <i>Stazeri</i> in millions per 1 g. of soil	Moisture in per cent	NO ₃ -nitrogen in mg. per 1 kg. of soil
15/VIII 32 (before irrigation)	0-5	0.3	4.8	84.0
	5-25	0.1	7.5	8.5
	25-40	0.2	7.9	12.2
19/VIII (on 16/VIII irrigated 1000 m ³ . per ha.)	0-5	0.8	20.6	116.5
	5-25	0.5	19.1	46.6
	25-40	0.4	16.1	9.5
21/VIII	0-5	3.0	17.0	246.0
	5-25	2.0	17.4	37.9
	25-40	2.0	16.5	21.1
27/VIII	0-5	3.0	5.9	86.7
	5-25	0.8	8.0	—
	25-40	0.4	9.1	3.0

The data thus obtained are summarized in Tables 3 and 4.

In all cases, after irrigation we observed an increase in the amount of denitrifiers. In comparing Tables 3 and 4 with Tables 1 and 2, we note that the behavior of the denitrifiers and the dynamics of the nitrates in the furrow are the equivalent of irrigation by submersion at the rate of 2000 m³., and in the

ridge to that at the rate of 500 m³. The phenomena of nitrate migration from the furrow to the ridge occurred, but if we are careful to calculate the total balance of nitrates, it will be negative. As a matter of fact, when there is a decrease of nitrates in the furrow from 304 mg. to 17 mg. per kg., we observe an increase in the ridge from 84 to 116 in the surface layer. The corresponding decrease in nitrates in the layers from 5 to 25 cm. thick in the furrow, from 42 mg. to 3 mg., results in an increase in the ridge from 8 to 46 mg. per kg. In other respects the dynamics of nitrates and the development of denitrificators correspond to the results of the experiment already mentioned above.

Thus, the problems of denitrification during irrigation certainly play a great part, independent of the kind of irrigation employed; only when irrigation is done by infiltration, the processes of nitrification and absorption of free nitrogen go on more energetically, and this leads to a more rapid accumulation of nitrates after irrigation.

III. Experiments in the regulation of the dynamics of microbiological processes by the use of antiseptics

In order to control this phenomenon of denitrification, we have performed a whole series of experiments in the use of antiseptics. As antiseptics carbon disulfide, a solution of dichlorobenzol in carbon disulfide and chlorpicrin were used. The experiments were carried out in soil under vines, under cotton in Gandzha, and under *Scorzonera tau-sagyz* in Bournoe. Different amounts of antiseptics were used, and they were applied at different dates. To avoid complicating our exposition by too many figures, we submit two tables — 5 and 6 — relating to the introduction of antiseptics in Gandzha under cotton in connection with irrigation, and Table 7 for Bournoe.

In 1932 experiments were made in Gandzha with the introduction of liquid ammonia in the shape of NH_4NO_3 , calculating 90 kg. of N per ha. as fertilizer, together with irrigation water on a basis of superphosphate — 90 kg. of P_2O_5 per ha.

For analysis soil was taken from plots fertilized with superphosphate and from plots where NH_4NO_3 and $\text{NH}_4\text{NO}_3 + \text{NH}_3$ were introduced with irrigation. The analyses are summarized in Tables 5 and 6.

As usual, after irrigation we observed an increase in the amount of denitrificators. The introduction of an antiseptic reduces their number. But this decrease continues for a comparatively short time. After 5 days their number has again attained its original amount.

We observed a more continued decrease of the number of denitrificators when using chlorpicrin in Bournoe on soil under «tau-sagiz».

Table 5

Experiment with introduction of nitrogenous fertilizers with irrigation. Ridge of the furrow (Gandzha).

Date	Plot	Denitrifica- tors, group <i>Bac. Stau- zeri</i> in mil- lions per 1 g. of soil	Moisture in per cent	NO ₂ - nitro- gen	NH ₄ - nitro- gen aqu.+ abs.
				in mg. per 1 kg. of soil	
8/VIII 1932	Initial basis	2.0	17.8	43.6	16.7
	NH ₄ NO ₃	0.3	15.5	31.1	17.4
	NH ₄ NO ₃ + NH ₃	0.4	15.2	37.1	17.0
17/VIII	Initial basis	—	5.6	—	—
	NH ₄ NO ₃	—	3.2	—	17.3
	NH ₄ NO ₃ + NH ₃	—	4.8	—	14.4
27/VIII (on 22/VIII irrigated: 1000 m ³ . per ha.)	Initial basis	6.0	17.5	—	13.3
	NH ₄ NO ₃	3.0	16.3	—	15.0
	NH ₄ NO ₃ + NH ₃	2.0	18.8	—	11.6
2/IX (on 30/VIII irrig. with antisept. 20 cc. per 1 m ² .)	Initial basis	0.9	18.0	65.3	11.6
	NH ₄ NO ₃	0.8	11.1	58.3	9.5
	NH ₄ NO ₃ + NH ₃	2.0	14.7	54.9	9.4
5/IX	Initial basis	7.0	11.8	37.6	13.1
	NH ₄ NO ₃	6.0	6.7	91.3	24.3
	NH ₄ NO ₃ + NH ₃	0.6	5.7	43.1	10.6

Table 6

Experiment with introduction of nitrogenous fertilizers with irrigation. Furrow (Gandzha).

Date	Plot	Denitrifica- tors, group <i>Bac. S. ut- zeri</i> in mil- lions per 1 g. of soil	Moisture in per cent	NO ₂ - nitro- gen	NH ₄ - nitro- gen aqu.+ abs.
				in mg. per 1 kg. of soil	
17/VIII 1932	Initial basis	—	4.7	—	14.3
	NH ₄ NO ₃	—	3.2	—	18.4
	NH ₄ NO ₃ + NH ₃	—	3.9	—	13.9
27/VIII (on 22/VIII irrigated: 1000 m ³ . per ha.)	Initial basis	4.0	18.8	—	13.0
	NH ₄ NO ₃	0.7	14.3	—	15.8
	NH ₄ NO ₃ + NH ₃	5.0	14.0	—	12.2
2/IX (on 30/VIII irrig. with antisept- ics.; 20 cc. per m ² .)	Initial basis	0.6	9.1	86.4	10.6
	NH ₄ NO ₃	2.0	10.7	78.8	10.2
	NH ₄ NO ₃ + NH ₃	0.8	10.7	76.8	10.1
5/IX	Initial basis	2.0	8.1	56.2	14.2
	NH ₄ NO ₃	3.0	5.0	52.6	14.5
	NH ₄ NO ₃ + NH ₃	0.4	4.1	104.5	12.7

From Table 7 it appears, that on the 7th day, when the total number of microorganisms calculated on Petri plates exceeded the control, the amount of denitrifiers was only $\frac{1}{5}$ to $\frac{1}{10}$ as much as in the control. The introduction of antiseptics during the time of the experiment had little influence on the content of nitrates and organic matter in the soil.

Table 7

Experiment with introduction of chlorpicrin (Burnoe).

Date	Plot	Denitrifiers group <i>Bac. Denitrificans</i>	Saprophites	Moisture	NO ₃ -nitrogen	Oxidizability
		in millions per 1 g. of soil		in per cent	in mg. per 1 kg. of soil	
4/VIII 1931	Control	3.0	4.4	—	—	—
	Experimental plot	3.0	4.7	14	2.57	64.4
11/VIII (on 9/VII introd. chlorpicrin: 3 g. per m ² .)	Control	4.0	5.2	15.4	238	70
	Between holes	0.5	3.7	12.0	240	—
	Out of hole	0.3	2.9	15.1	245	76
16/VIII	Control	—	—	17.5	—	—
	Between holes	0.9	6.7	15.5	—	—
	Around the hole	0.4	5.4	16.7	250	—

At the Valuiki Experimental Station as well as in Gandzha-G. S. Zakharaova observed a considerable increase of denitrifiers after irrigation, and the introduction of antiseptics had little influence on the development of the denitrifier.

Data concerning the use of antiseptics in the control of phylloxera show, that the conditions of temperature and moisture must be strictly observed. Thus, the problem of controlling denitrification by means of introducing antiseptics demands a still further careful research-work.

IV. Experiments in studying the causes of the low efficiency of nitrogenous fertilizers in the Frounze Region

In Central Asia one meets the phenomenon that in some regions the use of nitrogenous fertilizers was not profitable.

This phenomenon was observed, on the one hand, on light chestnut loams—soils poor in total nitrogen in the Frounze district, and, on the other hand, on «tougai»—boggy soils, near Tashkent. Our observations were made in both these regions.

The soils of the Frounze Zonal Experimental Station are light-chestnut loams, strongly alkaline, with a small content of humus: they are poor in nitrogen and have a considerable amount of total phosphorus. Nevertheless, according to

the data of the Frounze Zonal Experimental Station of the Scient. Research Inst. for Cottonplant Culture, these soils react well to the introduction of phosphate fertilizers and react very slightly to the introduction of nitrate ones.

Table 8

Yield of cotton-plant.

Forms of nitrogen on the background of phosphorus 90 kg. N and P_2O_5 (sum for three years).

	Centners per 1 ha.	In per cent
Control	23.02	100.0
$(NH_4)_2SO_4$	24.01	104.4
NH_4NO_3	27.37	118.9
Urea	27.19	118.1
Calcium cyanamide	24.85	107.5
Turkestan salpetre	28.55	124.0
Bisuperphosphate as background	26.40	114.7

As seen in Table 8, the introduction of $(NH_4)_2SO_4$ on the basis of phosphorus, in some cases even decreased the yield as compared with the initial soil.

The small efficiency of nitrate fertilizers may have resulted from two basic causes: first from the plant itself, if it was not able to take the nitrogen presented or did not need any surplus nitrogen as compared with the one present in the soil, and, on the other hand, from those transformations which the nitrate fertilizers might have endured in the soil itself when in contact with the microflora.

We stopped little on the state of the higher plant, but we assume that doses of 120—240 kg. of N in the form of NH_4NO_3 or $(NH_4)_2SO_4$ could not poison the plant by a too rapid introduction of ammonia because, according to the data of the Caunchi Experimental Station, the doses of 600 kg. of N in the form of NH_4NO_3 , in comparison with those lying lower, produced increments of cotton yields.

Another essential factor on the side of the higher plant—is the comparatively low absolute yield of cotton in the Frounze district. As a matter of fact, this region lies nearly at the Northern limit of cotton growth, where a small crop results because of a short growing period: and it is possible that for the given yield there is sufficient soil nitrogen.

As regards the activity of the microflora, it was necessary to elucidate 4 basic questions which are connected with the regulation of the nitrogen balance.

1. It was necessary, on the one hand, to make clear how strongly the process of fixing the free nitrogen goes on.

2. On the other hand, the nitrogen applied may be decomposed either a) from the activity of denitrificators, or b) because of the liberation of nitrogen in the shape of free ammonia, when introducing ammoniac salts, as the pH of the soil varying around 8.5.

3. May not nitrogen pass into a biological form, becoming thereby not available to the higher plant?

4. Does the following phenomenon take place: the introduction of considerable doses of ammoniac nitrogen, during its nitrification, does it increase the content of easily movable calcium in the soil at the expense of the formation of $\text{Ca}(\text{NO}_3)_2$ which fixes the free phosphoric acid; does it result in a decrease of yield or in the small efficiency of nitrogenous fertilizers introduced as a secondary phenomenon?

Part of the study was carried out at the Zonal Experimental Station of Frounze with the active participation of N. A. Shishelova, assistant at the Scientific Institute on Fertilizers. There we studied the actual state of separate groups of microorganisms on plots with the introduction of various fertilizers under cotton and opium poppy on irrigated and dry farming tracts. Further, the soil from control plots fertilized with superphosphate and ammonium nitrate was brought in a fresh state to Moscow and here subjected to experiments by Waksman's method modified by the Scientific Institute on Fertilizers (Yashnova, 1930) in order to ascertain the correlation between the separate groups of bacteria, and determine the intensity of the nitrification processes of ammonification and assimilation of free nitrogen. And, finally, a series of experiments was made in order to ascertain the possibility of chemically fixing the phosphoric acid as a result of the nitrification of ammonium nitrogen of the fertilizers being introduced.

In characterizing the current state of the microflora, besides soil analysis, we determined the numbers of denitrificators of the group *Bac. Denitrofluorescens* by Hiltner's method modified by the Scientific Institute on Fertilizers (Razumov, 1925), of *Bac. mycoides* by Koch's method on agar plates, the total amount of saprophites and in some cases the total count, of microorganisms, microscopically, by Germanov's (1932) method.

Soil analyses from plots under opium poppy (Table 9) relate to the period when the poppy had already finished growing, and all the changes in the chemism of the soil can be attributed only to the activity of microorganisms.

The analysis was made twice: July 29th and August 17th. The basic conclusions which it is possible to deduce from the data of the analyses can be summarized thus: 1) The total number of microorganisms is not great (on the control plot it remains below 1 milliard per 1 g. of soil) with a considerable amount of Azotobacter which attains 15% of the total number of bacteria.

Table 9

Opium Poppy.

Plot	Ho- rizon	<i>Bac. Mycoides</i>			<i>Bac. Deni- rofluorescens</i>	Saprophites	Total count	Azotobacter	Cocci	Rods	Moisture in per cent	NO ₃ -Nitr. in. mg. per 1 kg. of soil			
		in thousands per 1 g. of soil											in millions per 1 g. of soil		
29/VII 1933 Control	0-20	15	4 000	3 300	951	125	328	498	8.15	2.3					
	20-40	5	4 000	1 765	747	90	307	350	10.77	0.6					
P ₂ O ₅ -Superph. 90 kg./ha.	0-20	30	60 000	5 345	—	—	—	—	7.55	3.6					
	20-40	5	8 000	11 600	—	—	—	—	8.17	0.5					
NH ₄ NO ₃ -Nitrog. 90 kg./ha.	0-20	5	8 000	13 550	487	90	127	270	6.54	5.2					
	20-40	—	10 000	—	643	58	218	367	9.21	2.3					
Manure	0-20	10	4 000	10 700	—	—	—	—	7.94	3.8					
17/VIII Control	0-20	30	20 000	2 350	1 075	185	440	440	5.9	8.4					
	20-40	5	—	740	392	53	127	212	8.6	2.1					
P ₂ O ₅ -Superph. 90 kg./ha.	0-20	10	40 000	4 000	—	—	—	—	5.4	2.9					
	20-40	10	10 000	800	—	—	—	—	7.1	traces					
NH ₄ NO ₃ -Nitrog. 90 kg./ha.	0-20	10	40 000	4 920	249	27	111	111	5.9	12.6					
	20-40	10	7 000	6 000	270	32	111	127	7.6	10.4					
Manure	—	10	20 000	2 270	—	—	—	—	5.1	8.5					

On the plot fertilized with nitrogen the total number of micro-organisms is considerably lower than on the control; this last circumstance is an indirect indication of the fact that mineral nitrogen introduced is not transferred into protein nitrogen of bacteria bodies. 2) The amount of denitrifiers comes up in some cases to a considerable value of 60 000 000 per 1 g. of soil, especially on the plot fertilized with superphosphate, though soil moisture at that time reached only 7—8%.

Thus, on the basis of field observations we are able to answer three questions:

1. Fixation of free nitrogen goes on sufficiently energetically, this being indicated by a considerable amount of Azotobacter cells in irrigated and dry farming soils.

2. Denitrification can have a tangible significance, especially on tracts of land fertilized with phosphorus.

3. The nitrate fertilizers introduced are not transferred into a biological form to any considerable extent, because the total quantity of bacteria on the plots fertilized with nitrogen is less than on the control.

Laboratory determinations of soil biodynamics by Waksman's method modified by the Scientific Institute on Ferti-

lizers have in general confirmed the field observations and made possible the recording of a very great intensity of the nitrification process. Laboratory data have shown that considerable losses of nitrogen due to denitrification may occur when some easily assimilated organic matter is introduced simultaneously with the source of nitrogen. A certain increase of denitrifiers in the soil from the control plot and the plot fertilized with NH_4NO_3 was observed when ammonium phosphate was introduced. The introduction of phosphorus alone in the form of KH_2PO_4 had little influence on the state of the denitrifiers.

The question, as to the possibility of losses of ammonia nitrogen in the form of free ammonia, remained open, because, due to a great alkalinity of the soil, the latter could easily form from $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 and thus escape from the higher plant.

In order to solve this problem, soil with the addition of water, NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$ and urea reckoning 300 mg. nitrogen per 1 kg. was placed into Erlenmeyer's flasks. The soil was left in the thermostat at a temperature of 32°C . Air stripped of NH_3 was then drawn through the flask, and the forming ammonia was absorbed by Nessler's reagent.

The experiment lasted 7 days, and from Table 10 it may be seen that during the time of the experiment a negligible liberation of ammonia into the air was observed, when NH_4NO_3 and urea were added to the soil.

Table 10

Laboratory experiment for determining nitrogen losses in forms of ammonia at adding of nitrogenous fertilizers.

P l o t	Analysis of soil on the 10th day after starting experiment					Amount of NH_3 -nitrogen having been freed during the time of experiment. In mg. per 1 kg. of soil
	Mois- ture in per cent	NO_3 - nitro- gen	NH_4 - nitro- gen	P	pH	
Control	8	16.5	5.6	0.4	8.8	0.000
NH_4NO_3	9	228	39.2	0.44	8.7	0.004
Urea	9	105	67.5	0.57	8.5	0.008

From the table it is seen that, during the period indicated, a considerable portion of the ammonia nitrogen was transferred into the form of nitrates.

Thus, the problem of nitrogen losses in the form of ammonia or ammonium salts drops off.

The soils of the Frounze Zonal Experimental Station sharply react to phosphorus and contain a considerable amount of free CaCO_3 , from 3.5 to 11% by weight of dry soil. In view of this, Th. N. Germanov attracted our attention to the fact that, when introducing nitrogenous fertilizers into those soils in the form of ammonium salts or urea, a decrease in the content of easily soluble phosphorus may be readily expected. As a matter of fact, when introducing fertilizers the general biological activity of the soil is strengthened, and a considerable amount of carbonic acid is formed; the latter can easily transfer CaCO_3 difficult to dissolve into easily soluble calcium bicarbonate. Nitric and sulfuric acids, formed as a result of the intensely proceeding process of nitrification, act in the same way.

The presence in the soil solution of an easily soluble salt of calcium will transfer the phosphate salts, in the presence of alkaline soil reaction, into difficultly assimilated tricalcium phosphate.

We decided to approach the examination of this question by applying the method of spontaneous cultures, using the *Azotobacter* as a reagent for the readily assimilable phosphorus.

The soil was taken from the previous experiment, 0.25% mannitol and *Azotobacter* culture — 2 000 000 cells per each g. of soil, were added to it. The results of the experiment are summarized in Table 11.

Table 11

	Without addition of P	With addition of P	NO_3 -Nitrogen in mg. per 1 kg. of soil	NH_4 -Nitrogen
Control plate	220 colonies	The whole plate covered with a thick film of <i>Azotobacter</i>	16.5	5.6
NH_4NO_3	55 colonies	Growth weaker, but colonies merge	228	39.2
Urea	4 colonies	Separate colonies	105	67.5

When we compared the control plot with the plot fertilized with ammonium nitrate, a similar picture was observed (Table 12).

Control analyses showed that the addition of nitrate nitrogen to soil in doses of 240 mg. per 1 kg. in the presence of phosphorus only slightly oppresses the development of *Azotobacter* as compared with the control soil, so that it cannot be said there is any weakening of *Azotobacter* growth at the cost of a great amount of nitrates in the previous experiments; it is also seen from Table 11 that on plates with soil+urea the growth of *Azotobacter* went on more weakly though there were less nitrates, than in the soil with an addition of NH_4NO_3 .

Table 12

Plot	Amount of colonies on plate soil+mannitol without addition of phosphorus	NO ₃ -Nitrogen	NH ₄ -Nitrogen
		in mg. per 1 kg. of soil	
Control	520 colonies	8.5	2.7
NH ₄ NO ₃	65 colonies	38	2.8

Control experiments with the addition of soluble salts of calcium in field doses did not provide any sharp decrease of the amount of Azotobacter colonies, as compared with nitrate. Increased doses of CaCl₂ corresponding to the amount of Ca(NO₃)₂, which might form with a dose of nitrate equal to 240 mg. per 1 kg., noticeably hindered the development of the Azotobacter.

It thus seems that the question set forth by Th. N. Germanov about the indirect decrease of the content of easily soluble phosphorus by introducing considerable doses of nitrogenous fertilizers into soils poor in phosphorus and rich in CaCO₃ can have a great importance.

Data of cotton yields also indicate that when urea and ammonium sulfate are introduced, a lower yield is observed than after Turkestan saltpetre (Table 8). This conforms well with the above mentioned considerations, because when introducing KNO₃ we can expect a formation of half the easily soluble salt of calcium, as compared with that which would form when introducing urea or ammonium salts.

Summing up the above stated facts, the following points may be noted:

1. Fixation of atmospheric nitrogen on the soils of the Zonal Experimental Station goes on with sufficient energy.

2. The soils contain a considerable amount of denitrifiers, up to 40—60 millions per 1 g. of soil. So that during irrigation considerable amounts of nitrates can be decomposed to free nitrogen.

3. On the basis of laboratory experiments no losses of nitrogen in the form of NH₃ when introducing ammonium salts and urea into the soil is observed.

4. The small efficiency of fertilizers cannot be explained at the cost of the transfer of nitrogen into a protein form in bacteria bodies.

5. The introduction of considerable doses of nitrogenous fertilizers may partially decrease the yield in an indirect way, lowering the amount of easily soluble phosphorus in the soil solution and, finally, the plant may suffer from lack of phosphorus. However, this last question demands further investigation.

V. Experiments for studying the causes of the small efficiency of nitrogenous fertilizers in the district of Tashkent

In order to examine the poor efficiency of nitrogenous fertilizers on «tougai» soils near Tashkent, stationary experiments were carried on by I. I. Dobrogaiev under «kendir» on plots with introduction of ammonium sulfate and superphosphate. Samples were taken 3 times in the second half of the vegetational period and, as an example, data of one analysis are here quoted. The analyses were made in the usual way. The scheme of the experiment is seen in Table 13.

Table 13

Dynamics of microbiological processes under «Kendir» with introduction of various fertilizers. Tashkent.

Plot	Horizon	Denitrificators in millions per 1 g. of soil	<i>B. Mycoides</i> , thou- sands per 1 g. of soil	Moisture in per cent	NO ₃ Nitro- gen	NO ₂ Nitro- gen	NH ₄ Nitro- gen	Oxidizability in mg. O ₂
					in mg. per 1 kg. of soil			
2I/VIII Control	0—20	60	3.3	40.7	213	3.8	15.5	267
	20—40	100	3.3	47.8	26.7	3.8	8.1	179
(NH ₄) ₂ SO ₄	0—20	40	10	51.4	117.9	5.7	18.2	276
	20—40	60	6.6	47.8	10.1	0.2	9.1	201
Superphosphate	0—20	50	3	63.5	118.7	16.3	10.2	—
	20—40	20	3	41.5	6.5	0.56	4.8	148
(NH ₄) ₂ SO ₄ +Su- perphosphate	0—20	50	3	46.1	115.2	4.48	13.5	226
	20—40	40	16.6	43.3	23.2	0.76	11.2	253

It is necessary to note here the exceedingly high moisture content of the soil due to a high level of ground waters and a very great amount of denitrificators averaging 40—50 millions to 1 g. of soil with high values of organic matter soluble in water. The presence of nitrates under these conditions provides the possibility for denitrification processes to go on energetically. As seen from Table 13, the plots into which were introduced the fertilizers contain a smaller amount of mineral nitrogen than the control plot.

Further analyses of Table 14 have shown that the amount of denitrificators on fertilized plots remains all the time higher than on the control plot; due to this a smaller amount of nitrates is also observed on the fertilized plots; as the «kendir» on those plots in 1931 was poor, the decrease of nitrates cannot be attributed to the activity of a higher plant. Hence the small effect of nitrogenous fertilizers on «tougai» soils at the high level of

Table 14

Dynamics of microbiological processes under «kendir» with introduction of various fertilizers. Tashkent.

Plot	Horizon	Denitrifica- tors in mil- lions per 1 g. of soil	<i>B. Mycoides</i> in thousands per 1 g. of soil	Moisture in per cent	NO ₃ - Nitro- gen	NO ₂ - Nitro- gen	NH ₄ - Nitro- gen absor.	Oxidizabili- ty in mg. O ₂
					in mg. per 1 kg. of soil			
19/IX Control	0—20	30	1	44.4	132.7	2.5	6.7	364
	20—40	3	1.6	48.0	25.4	4.4	9.4	382
(NH ₄) ₂ SO ₄	0—20	60	18.3	56.4	117.0	3.8	9.0	269
	20—40	40	23.3	46.3	3.2	4.3	7.5	198
Superphos- phate	0—20	80	3.3	46.6	82.1	3.1	6.9	313
	20—40	60	26.6	38.7	3.3	1.1	5.7	108
(NH ₄) ₂ SO ₄ +su- perphosphate	0—20	70	3	47.6	101.8	0.2	7.6	367
	20—40	80	43.3	42.7	8.7	5.2	6.4	320

ground waters is closely connected, in our opinion, with the process of denitrification.

Observations were also carried on there by Dobrogaiev on microflora in connection with the depth of the standing of ground waters on plots under «kendir», «kenaph» and the Rope-plant; the microflora was studied in the nursery and on plantations of «Rami». I will not dwell longer on this and will only note that the amounts of denitrificators were everywhere near to those mentioned above.

VI. Study of the dependence of the Azotobacter distribution on irrigation

M. M. Cononova in her study: «Microbiological soil characteristics of some regions of Central Asia» (1930) establishes a determined connection of the Azotobacter's expansion and activity with irrigation. According to her data, though Azotobacter is also found on unirrigated soils, it grows on silica gel plates only in the case when some mannitol has been added to the soil, the soil having been moistened and kept in this state for a fortnight. Because of the appearance of rapid growth of Azotobacter on silica gel plates of soil samples taken from plots subjected to irrigation, Cononova affirms that active Azotobacter is brought only with irrigation waters. Microscopical analyses of the amount of bacteria in the horizons of dry farming soils of the Frounze Zonal Experimental Station (Table 15) and of soil-grounds of virgin soil from the Reserve of the Dzhaifarkhan Solonchak (Saline) Experimental Station in Mogan (Table 16) show a considerable per cent of Azotobacter cells which extended to considerable depths, as it is easy to see from the tables.

Table 15

Distribution of bacteria along horizons on a dry-farming plot under cotton (Frounze).

Horizon	Azotobacter	Cocci	Rods	Total amount
	in millions per g of soil			
0-20	121	328	227	676
20-40	79	291	153	523
40-60	52	148	142	342

Table 16

Distribution of bacteria along horizons on a virgin soil plot (Mugan, Dzhaferkhan Experimental Station).

Sample taken at the depth	Azotobacter	Cocci	Rods	Total amount	Description of soil profile
	in millions per 1 g. of soil				
0—14	280	490	670	1 470	Heavy loam grey brown
25	49	558	664	1 271	Heavy loam brown
36—37	0	1 082	346	1 428	Grey sand
45	0	1 032	550	2 582	Medium loam, dark, buried horizon
70	0	648	317	965	Yellow-grey sandy loam
100	0	414	332	746	Grey sand

The problem stands before us as to whether silica gel plates are a sufficient criterion of Azotobacter activity.

Do we not meet here the same phenomenon which was observed by Dianowa and Woroschilowa (1930) on the fields of the Timiriacheff Academy of Agriculture when they denied the presence of Azotobacter cells in soils from plots manured with stable manure and identified the microscopically determined microorganism with the one similar to Azotobacter?

The method of discovering the Azotobacter applied by us was the same as the one used in their study by Nowogrudsky and Naumowa (1932) when they were able to prove the presence of Azotobacter on those plots where Dianowa and Woroschilowa found none. As a matter of fact, when we set spontaneous cultures with drainage we were able easily to obtain separate Azotobacter colonies on dry farming soils of the Frounze region and on the virgin soils of Mugan and Valuiki.

A further isolation of the organisms out of these colonies on agar media has shown that we have to deal with a typical Azotobacter.

It is thus possible to establish with surety that the Azotobacter is present in dry farming lands and that its expansion is not connected with irrigation waters.

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BIOLOGY AND BIODYNAMICS OF SALINE SOILS (SOLON-CHAKS) ALKALI SOILS (SOLONTZI) AND SOLODIZED SOILS

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Saline, alkali and solodized soils and solodis, being soils genetically connected with each other, markedly differ one from the other in their fundamental properties. These soils, being different stages of one and the same soilforming process, acquire in their evolution a series of properties which create differing conditions for soil organisms, modifying in this or other way the biology and biodynamics of these soils. The high concentration of salts of the soil solution of saline soils represents for soil organisms another medium than the alkali soils, the soil solution of which contains a normal concentration of soluble salts, but the properties of which are determined by the presence of absorbed sodium in their absorbing complex; it is the sodium absorbed that creates the high alkalinity of these soils, as well as their high colloidalilty in moist condition. Solodized soils and the solodis, being the next stage of the alkali soils' evolution, contain neither high concentrations of soluble salts in the soil solution, nor absorbed sodium in their absorbing complex, and the reaction of these soils is neutral or slightly acid, yet, resulting from the natural process of the «disalkalization» of alkali soils, the latter have lost their most active, the most valuable for plants and microorganisms, organic colloidal part which makes these soils a different medium for soil microorganisms. The evolution of these soil properties, their transformation from saline soils through those alkali into solodis, is one of the most interesting problems of soil science. Another problem of no lesser interest should be that of the alteration of the biology of these soils in the course of the natural process of their dissalinisation, disalkalization and solodisation. This problem, apart from being of a great theoretical interest, is also one of a great practical importance, being in direct connection with problems of the reclamation of these soils.

Our knowledge of the nature of alkali and solodized soils dates but from recent times. The work of the member of the

Academy K. K. G e d r o i z (21), having disclosed the essence of alkali soils as well as in that they differ from saline soils, was published in 1912, whilst his work (22) on solodized soils and solodis refers to 1921. Up to that time this problem of soil science was rather obscure, and the conceptions of a «solonchak» (saline) or a «solonetz» (alkali soil) was often not distinguished and did not correspond to the meaning we attach to these conceptions at the present time. This told in particular on works of a microbiological character, in which it was often difficult to establish, even in works of recent years, what was the soil the author had to deal with, there generally being absent in these works the chemical and morphological characteristics of soils from which samples had been taken; yet, even where such a desultory characteristics of the examined sample is quoted, just doubts would often arise, as to the correct denomination of these soils. All this creates a special difficulty for the summarizing of the literary material on the problem of the evolution of soil biology in connection with the evolution of soils from saline soils to solodis.

The second moment, depriving us of the possibility to fully elucidate this problem, is the comparatively small number of investigations carried out for the study of the biology and biodynamics of soils we take interest in.

Saline and alkali soils, being an unfavourable medium for the development of higher plants, the former — in virtue of the high concentration of salts, the latter — due to their high alkalinity, present a favourable medium for the growth of lower vegetable forms and bacteria. According to B. A. K e l l e r's researches (1), saline and alkali soils of deserts and semideserts where higher forms of the vegetable kingdom are almost absent, are abundantly populated with lower plants; of these bluegreen algae, lichens, liver-worts and foliated mosses — develop most abundantly upon crusty-columnar and deeply-columnar alkali soils. M. L. S t e p a n o v a (14) cites high figures for the bacteriological population of saline and alkali soils, higher than those of all other soils she had studied. According to B. A. K e l l e r's and A. F. K a r e l s k a y a's (3) researches the columnar and crusty-columnar alkali soils, in the environs of the station Toida, in the North-Eastern part of the Voronezh gov., are much more densely populated with bacteria than chernozems and slightly podzolised soils of the same district. T. N. G e r m a n o v (9, 10) denotes in his works too the alkali soils of the Dnieper's ice-lobe to be more densely populated as compared to normal chernozems of the Kharkov gov. P. A. H e n k e l and N. D. Z a c h a r o v a (18) also cite high figures of the density of bacteria populating alkali soils they had studied. One may say, on the ground of literary material, that saline and alkali soils represent, by their properties, a favourable medium

for soil microorganisms, and this is equally true of alkali and saline soils of Southern, as well as of Northern zones; while solodized soils and solodis, which are the next stage of the evolution of these soils, are markedly inferior to salty and alkali soils, likewise to the other normal soils, in density of the population of microorganisms. Thus, according to data of T. N. Germanov (8, 9, 10), the total quantity of bacteria in the solodized chernozem of the Nossovka Exp. Station proved to be lower than in other soils, when it was comparatively studied together with alkali and normal soils of the same locality.

Data we possess, relatively to the qualitative composition of the microflora of these soils, show a great diversity in the micropopulation of saline and alkali soils. G. Burgwitz (13) points out, in his research of the group composition of the microorganisms of certain Russian soils, that «the saline immature soil of the coasts of the Caspian Sea possesses a rich and varied bacterial flora». *Azotobacter*, the nitrifying, denitrifying and cellulose destroying bacteria in these soils are denoted to be in a great quantity, *Clostridium Pasteurianum* and putrefactive bacteria being found in a lesser quantity. M. L. Stepanova (14) notes too the great diversity of the forms of soil microorganisms in saline and alkali soils. One may say that the basic groups of soil microorganisms being of importance for agriculture, are present in these soils, finding in them favourable conditions for development. Solodis and solodized soils present somewhat different conditions for the development of these groups of bacteria; some of the agricultural groups may be absent in these soils, or exist in a stunted condition. One could not succeed, according to T. N. Germanov's (7) investigations, in isolating certain groups of bacteria from the solodized chernozem of the Nossovka Exp. Station; others, as for inst., the *Azotobacter*, were found in a stunted state.

Most of investigations on the biology and biodynamics of the soils we are interested in, have been directed to studying in these soils of those groups of soil bacteria which are important for agriculture; much attention has been paid in that respect to nitrogenfixators and nitrificators. A great many authors show the *Azotobacter* to be found in great quantities in saline and alkali soils. Thus, A. F. Karelskaya (3, 5) adduces data on the great quantitative development of the *Azotobacter* and *Clostridium* in alkali soils, the former being absent in a podzolised soil and in thick chernozem. T. N. Germanov (9, 10) brings forward in his researches, being a comparative study of the nitrogenfixing capacity of alkali and solodized soils, high figures of the quantity of the *Azotobacter* found in alkali soils, being much higher than in solodized soils, or even in normal chernozem. D. A. Sabinin, and P. A. Henkel (6) conjointly with N. D. Zacharova (18) have also noted

in their researches the intense development of the *Azotobacter* in alkali soils, where it is most often to be met, and its being in the least quantity, or even quite absent, in solodis and podzols. A great many indications on the high number of the *Azotobacter* in saline and alkali soils may be found in the Transactions of the Section of Agricultural Microbiology to the State Institute of Experimental Agronomy. Hence, the high development of the *Azotobacter* and of other nitrogenfixators in saline and alkali soils, and small density of the *Azotobacter* population, or even its complete absence in solodis and solodized soils, may be considered as being a fact more or less proved.

Thus, saline and alkali soils, according to data we possess, constitute a medium extremely favourable for the life and activity of nitrogenfixators and, in that respect, prove to be soils of a high nitrogenfixing capacity. A series of investigations give us a quantitative expression of this process in the mentioned soils.

Works of A. F. Karelskaya (3, 5), T. N. Germanov (8, 9, 10), V. Beresnev and O. Shvetzova (11), D. A. Sabinin and P. A. Henkel (6), P. A. Henkel and N. D. Zacharova (18), and of other authors mark the high nitrogenfixing capacity of alkali soils, as compared to that of other soils. It is interesting to note, relatively to this, that solodized soils and solodis, having developed from alkali soils and, seemingly, having possessed a high nitrogenfixing capacity when being in the stage of alkali soils, have lost this capacity in the process of a natural disalkalization, in virtue of the alteration of a series of their properties, i. e. that the natural process of disalkalization has caused the formation of soils, being no more a medium favorable to the development and activity of the *Azotobacter*. T. N. Germanov's (9) comparative study of the nitrogenfixing capacity of alkali soils with other soils of the same locality, has shown even those alkali soils, in which the process of degradation had penetrated rather deeply, to contain great quantities of the *Azotobacter* and to possess a high nitrogenfixing capacity, as compared to that of adjacent solodized chernozems, possessing it already in a very insignificant degree. Such a pronounced difference in the nitrogenfixing capacity of alkali and solodized soils, soils genetically connected with each other, draws our attention to elucidating the causes creating unfavourable conditions for the development and activity of nitrogenfixators in these soils. This is impossible to be explained by the alteration of the reaction in these soils; normal chernozems, the reaction of the soil solution of which very nearly approaches that of alkali and solodized soils, possess a sufficiently high nitrogenfixing capacity. It is, seemingly, the consequence of a disintegration (taking

place in the process of a natural disalkalineness of alkali soils) of the absorbing complex of the soil, and of the loss of its humate part, being as well the consequence of the small mobility of the organic matter remaining in soil, in virtue of the removal at the process of disalkalineness of the absorbed sodium from the soil absorbing complex, which creates a deficiency in the energetic substance in soil for nitrogenfixators. The latter has been confirmed by the non-published as yet investigations of the Nossovka Exper. Station, as to the problem of the causes of the low nitrogenfixing capacity of the solodized chernozem of the Nossovka Station.

The study of the processes of the mineralisation of the organic nitrogen in saline, alkali and solodized soils indicates as well the high ammonifying and nitrifying capacity of saline and alkali soils, as likewise the decrease of these processes in solodized soils. M. S. K u z m i n (16) has noted in his works a considerable accumulation of nitrates in the columnar alkali soil at all the periods of observation, a much greater one than in the Southern chernozem. A. A. R i c h t e r's works point out too the high nitrifying capacity of the alkali soil he had been studying. E. V. B o b k o, N. P. O s t c h e p k o v and N. I. B e l k i n (15) quote data showing a much more intensive formation of ammonia and nitrates in a columnar alkali, as compared with the formation of these forms of nitrogen in the Southern chernozem. The same may be found in works of A. A. O b r a s t z o v a (17), which show the nitrification capacity of the alkali soil to be the double of that of chernozems. We find somewhat different data on the nitrifying capacity of the alkali soil in V. B e r s e n e v a's and O. S h v e t z o v a's (11) works, as well as in the investigations of G. L o p a t i n a (18), who consider the nitrifying capacity of alkali soils to be somewhat lower than that of chernozem, and much lower than that of saline soils the latter exceeding, to a considerable extent, the two other soils in the capacity to form nitrates. Naturally it is difficult to expect saline and alkali soils, taken in different regions, to possess equal biological and other properties, therefore it is but natural that a certain discrepancy exists in findings of different authors relatively to the nitrifying capacity of these soils. But it is clear that saline and alkali soils according to their properties are a quite favourable medium for ammonifying and nitrifying bacteria, and processes of nitrogen mineralization in these soils take their course with sufficient energy.

Not the same is to be seen in solodized soils; as a result of the natural process of disalkalization, these soils acquire a series of properties, negatively influencing the life and activity of nitrifying bacteria, in consequence of which there occurs in these soils a stunting of the process of nitrification and a feeble potential nitrifying capacity, as it may be seen from data in the

works of V. G. Taranovskaya and T. N. Germanov (7, 23), and of N. N. Sushkina (19).

Thus, two fundamental biological processes, the most essential for agriculture, take their course differently in soils under our examination: saline and alkali soils are biologically highly active soils, whereas the biodynamics of solodized soils and solodis is stunted, due to specific properties of these soils acquired in the process of their genesis, and these soils offer a less favourable medium for the life and activity of basic agricultural groups of soil bacteria; the total density of microorganisms populating them is lower than in saline and alkali soils.

Solodized soils and solodis are not only biologically feebly active, but chemical and physico-chemical processes take their course in them with lesser energy than in other normal soils (K. K. Gedroiz — 7); one may say, that the energetics of these soils is noticeably lowered as compared to other normal and alkali soils. The cause of this is to be looked for in the genesis of these soils, in the process of the natural disalkalization of alkali soils, bringing to the formation of solodized soils and solodis. This process results, on the one hand, in a loss, through the leaching out, of the most dispersed, humate part of the soil colloid, and, on the other, a disintegration takes place up to the terminal products of the downbreak, under the influence of water of the aluminosilicate part of the absorbing complex of these soils. Both these facts have for result a decrease in soils of the colloidal fraction, and, consequently, a decrease of the specific surface of these soils, as well as a lowering of the surface energy. We may see in how much solodized soils have got poorer in the colloidal fraction from data adduced in Table 1, obtained by the agrochemical section of the Nossovka Exp. Station when studying the problem of the properties of solodized soils, as compared to other soils of the forest-steppe in the Ukraine.

Table 1

Soils	Colloidal fraction in % of total amount of soil	Total soil surface in hectares ¹
Chernozem of the Kamenno-Steppe Exp.St.	34.96	13386
» » Krasnograd » »	28.71	10921
» » Sumy » »	18.12	—
» » Nossovka » »	8.75	3556
» » Belotzerkov » »	13.77	5359
Loam of the Poltava » »	19.88	—

¹ The total surface of soil has been computed after data of the ultramechanical composition, to a layer 0—10 cm. thick of an area of 1 hect. Data have been expressed in thousands of hect.

We see the solodized chernozem of the Nossovka Station to possess a much lesser colloidal fraction, and the total surface of the solid phase of this chernozem to be much smaller, than the

total surface in other soils. Consequently, the energy of this soil surface, conditioning processes and phenomena taking place in the dispersed phase of soil, as well as processes of the interaction of the solution and the solid part of soil, have to take their course with less energy than in other soils. The lowered physical and physico-chemical capacity of this chernozem should not, however, be considered the principal cause of its decreased biological activity and of the low yielding capacity of this soil (14, 25). One more reason for the solodized soils and solodis to present an unfavourable medium for microorganisms is that, in virtue of their having got poor in humate colloids, which are an energetic material for most of the soil microorganisms, the nutritive value of these soils is strongly lowered, whilst in alkali soils, due to the presence in their absorbing complex of absorbed sodium, soil colloids are yet in a dispersed condition, most accessible to their being affected by a soil solution, which raises the nutritive qualities of these soils for soil microorganisms.

The natural process of disalkalization brings thus to the formation of soils of low activity. K. K. G e d r o i z writes as follows when touching on the necessity of preserving the absorbing complex of the soil: «This complex presents the most valuable part in soil, and, proportionally to its disintegration, soil transforms more and more from an aggregate of very complex and comparatively unsteady compounds, conditioning its life and its fitness to be a medium for the life of plants and microorganisms, into a mixture of simple and stable compounds, i. e. into a dead body». All the above said makes problems of the reclamation of saline and alkali soils be to central in our attention, and prompts us to widen and endepen our knowledge on the biology of these soils.

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REVIEW OF WORKS ON ROOT NODULE BACTERIA OF THE INSTITUTE OF AGRICULTURAL MICROBIOLOGY

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The capacity of nodule bacteria of leguminous plants to assimilate nitrogen, in this or other degree depends, first of all, on the peculiarities of individual strains. M. L ö h n i s and other authors consider certain strains of nodule bacteria not to be capable of assimilating nitrogen, yet no such strains were met in the course of our works¹ among a considerable number of strains of *Rhizobium* from different leguminous plants pertaining to different groups. All of the strains examined, capable of forming nodules, were able too to assimilate nitrogen. However, as may be seen below, any of these strains may not, practically, play any rôle in the accumulation of nitrogen by the leguminous plant, under conditions of a definite nitrogen regime of the latter, in spite of an abundant formation of nodules. It is but a detailed and close study of the correlations of the complex formed by nodule bacteria, leguminous plant and soil, that may give the possibility of penetrating deeper into conditions necessary to nodule bacteria for effectuating an energetic nitrogen fixation. The problem of the activity of individual strains, relatively to nitrogen assimilation, continues to be one of the most interesting and, at the same time, practically important problems, on a line with the problem of obtaining nitragin of the maximum efficiency. The problem, not solved up to now, of the rôle nodule bacteria of morphologically different stages of development play in fixation of nitrogen — is also of an extreme interest.

Connected with the main problem mentioned above, investigations were carried out on the following subjects: 1) assimilation of free nitrogen by pure cultures of nodule bacteria, 2) methods for preparing concentrated preparations of nitragin, 3) elucidation of the method for determining the most active strains of *Rhizobium*, in respect to nitrogen assimilation, 4) the course of nitrogen accumulation in leguminous plants and the participation in this accumulation of the different stages of the bacteria development, and 5) dependence of the nitrogen assimilation

¹ This work was carried out at the All-Union Institute of Agricultural Microbiology in Leningrad.

lating activity of nodule bacteria in nodules on the nitrogen regime of leguminous plants.

Assimilation of free nitrogen by pure cultures

Experiments, carried out relatively to the assimilation of nitrogen by pure cultures of different strains of nodule bacteria on liquid and solid media have shown pure cultures not to give any definite increase in nitrogen. This increase is either absent or does not exceed 0.9—1.0 mg. which is a value not allowing to come to any conclusions. Determinations of the consumption of glucose, performed together with determinations of the nitrogen assimilation, may be seen in Table 1.

Table 1

Rhizobium strains	Conditions of experiments	Medium with a 2% glucose	Time-limits of test	Consumption of glucose in g.
Soya-bean, Tokio	Sandy cultures	Mineral solution (without N)	27	0.11
	Same cultures	Mineral solution + $(\text{NH}_4)_2\text{HPO}_4$	27	0.17
	Liquid medium Layer 1.3 cm. thick	Mineral solution (without N)	27	0.18
	Same	Mineral solution + $(\text{NH}_4)_2\text{HPO}_4$	27	0.18
Soya-bean, Wright	Same	Mineral solution (without N)	27	0.17
	Same	Mineral solution + $(\text{NH}_4)_2\text{HPO}_4$	27	0.19
Soya-bean, Tokio	Liquid medium	Mineral solution (without N)	30	0.015
	Same	Mineral solution with KNO_3 0.05%	30	0.17
	Same	Mineral solution with asparagin	30	0.5
	Same	Yeast water	30	0.19
	Same	Bean decoction	30	0.15
	Same	Extract from the soya roots	30	0.15
Clover	Same	Mineral solution + KNO_3 0.05%	22	0.09
	Same	Mineral solutions + KNO_3 0.05% + CaCO_3	22	0.10

¹ Compositions of the mineral solution; K_2HPO_4 — 0.5 g.; MgSO_4 — 0.2 g.; NaCl — 0.2 g.; 1 litre of water.

These experiments have shown that bacteria in most various media very faintly assimilate carbohydrates, consuming at the

same time glucose in the quantity of 0.15—0.19 g. during one test. This consumption of glucose proved to be still lower in the absence of sources of nitrogen in certain tests. Seemingly, the introduction of sources of nitrogen raises to a certain degree the development of nodule bacteria, though not giving them the possibility of displaying an intensity of growth sufficient for a considerable consumption of carbo-hydrates to take place. Works of S. P. Kostytshev and S. I. Winogradsky have established the process of the assimilation of free nitrogen by the *Azotobacter* to take course through the formation of ammonia and, consequently, to be an exothermic process, not requiring the consumption of energetic material. There is no reason for supposing that the process of the assimilation of nitrogen by the *Rhizobium* should take another course. It has been elucidated, in respect to the *Azotobacter*, that there exists a definite correlation between the quantity of the assimilated nitrogen and that of the consumed carbo-hydrate, despite that the formation of ammonia, at the expense of free nitrogen, does not require the consumption of energetic material; the amount of assimilated nitrogen per 1 g. of consumed carbo-hydrates does not surpass, as a rule, 10 mg., i. e. there occurs a considerable expense of energetic material. This fact has to be explained by that the very assimilation of nitrogen, though being an exothermic process, cannot take place otherwise than under the condition of the development of bacteria, which requires a considerable consumption of carbo-hydrates. Assimilation of nitrogen by the *Azotobacter* cannot take place without consumption of carbo-hydrates; evidently, the same should be referred to nodule bacteria. Experiments summarized in Table 1, show the consumption of glucose by nodule bacteria to take place under most various conditions of experiments, with different sources of nitrogen, mineral as well as organic, in most insignificant doses; consequently these bacteria develop with insufficient energy. The absence of nitrogen assimilation in pure cultures is, thus, explained by nodule bacteria not being able to develop with sufficient energy under conditions under which tests had been carried out up to now, and not by their being deficient in the ability of assimilating nitrogen outside a leguminous plant. The consumption of carbo-hydrates by nodule bacteria may serve for measuring their development. Seemingly, the problem of the ability of pure cultures of nodule bacteria to assimilate free nitrogen will be solved only when favourable conditions will be found for their growth outside a leguminous plant.

Methods for preparing nitragin

Nitragin being widely applied in practice, an elaboration of standard methods is required for the preparation of the most effective nitragin. It is necessary, first of all, to dwell on the

problem of what should be the criterion of the quality of nitragin. Tests carried out by the Institute for Agricultural Microbiology have shown that the percentage of the infection of plants influenced by nitragin, and in certain cases, even the rise of yield, cannot be taken as such a criterion. At a 100% infection of the leguminous plants, the number of nodules on an individual plant, incapable of a natural contamination from soil, is often so insignificant (4—6 nodules) that such an infection cannot produce any increase in yield; on the other hand, as it will be seen further, even a maximum infection does not tell on yield on certain soils, fully supplied with nitrogen (Table 9). In connection with this, the criterion of nitragin activity should be considered as a combination of two features: percentage of infected plants and number of nodules upon them. Connected with the necessity of elaborating standard methods for preparing nitragin, on the one hand, and, on the other, with it being desirable to obtain preparations giving the maximum of contamination, the Institute for Agricultural Microbiology has set itself the aim to approach problems of preparing of nitragin by way of the application of a quantitative criterion. A thorough checking of the pureness of cultures has firmly taken root in the practice of preparing nitragin while methods of a quantitative line of approach to its preparation and estimation have not been elaborated at all. When preparing nitragin, the inoculation of the media—usually sterilized soil—has to be carried out by liquid cultures of nodule bacteria, the latter, however, do not give always a particularly strong development in liquid media, so that the nitragin obtained does not contain a sufficient quantity of root nodule bacteria per 1 g. of soil. The problem of obtaining effective nitragin may be approached in two ways: either by that of introducing, when preparing it, greater quantities of bacteria, or by that of creating such conditions, under which nodule bacteria might propagate in the preparation itself. Nitragin of a maximum efficiency is not difficult to be obtained under laboratory conditions: any concentration of the content of bacteria in preparation may be attained at the inoculation in a sterilized soil of an emulsion washed off from solid media. Another picture is to be seen at mass preparation. A direct calculation of bacteria under microscope, after Winogradsky's somewhat altered method, showed about 150—200 millions of root nodule bacteria to be found per 1 g. of soil in our laboratory nitragin, whereas the quantity of bacteria in nitragin of a mass preparation was approximately but 1 million per 1 g. of soil.

A very strong contamination was obtained at the application of such concentrated nitragin, for instance, to cultures of soya-bean plants in field experiments in the Northern Caucasus, where soya-bean plants do not yield nodules unless they are inoculated

with nitragin; thus, more than 200 nodules were numbered on one plant (Table 9).

Quantity of bacteria in millions per 1 cc. of liquid. 1.3 millions entered per 1 cc.

Table 2

Time limit in days	Rhizobium strains	Soya-bean decoction	Bean decoction	Yeast medium.	Mineral medium № 1	Mineral medium. № 2
3	Alfalfa	24	—	2	24	6
6	»	141	—	50	131	136
11	»	160	—	101	200	210
4	Soya-bean gelb	44	44	1	16	2
7	»	52	52	4	32	50
12	»	82	82	1	121	154

¹ Yeast prepared after the Windisch method.

Table 3

Rhizobium strains	Time limit in days	Yeast ¹ hydrolysate diluted 25 times		Yeast ¹ hydrolysate diluted 70 times		Yeast ¹ hydrolysate diluted 140 times		Bean decoction 10%	Bean decoction 1 1/2%
		With 0.5% of sugar	Without sugar	With 0.5% of sugar	Without sugar	With 0.5% of sugar	Without sugar		
<i>Lupinus angustifolius</i>	3	4	4	46	54	—	—	44	—
»	6	7	31	82	124	—	—	52	—
<i>Trifolium incarnatum</i>	3	18	55	84	90	—	—	15	—
»	6	23	78	153	164	—	—	38	—
<i>Lupinus angustif.</i> inoculated 0.1 mill. per cc.	3	—	—	14	26	3	4	38	4
<i>Lupinus angustif.</i> inoculated 0.1 mill. per 1 cc.	6	—	—	170	176	36	30	52	20
<i>Lupinus angustif.</i> inoculated 0.5 mill. per 1 cc.	3	—	—	45	57	15	42	42	28
<i>Lupinus angustif.</i> inoculated 0.5 mill. per 1 cc.	6	—	—	175	198	90	88	76	76
<i>Trifolium incarnatum</i>	3	—	2	83	200	56	90	88	2
»	6	—	—	170	360	80	160	120	5

¹ Yeast hydrolysate applied in experiments given in Table 3 was called — hydrolysate of yeast, prepared after D nee per and Gil we r, with pH-7.

Having in view that only liquid media are suitable for mass production of nitragin, experiments were carried out, by means of a quantitative method, for finding out liquid media most fit for nodule bacteria to be developed in; bacteria were

counted in a liquid medium by means of a direct method under microscope. Some of the synthetic media were also tested; the latter are more advantageous as compared to different extracts, owing to their composition being perfectly constant, whilst that of the usually applied decoctions may vary in a greater or lesser degree according to the character of the material.

Hydrolysate of yeast, obtained by way of yeast hydrolysis in an acid medium, was applied in experiments shown in Table 2; hydrolysate of yeast, prepared by way of hydrolysis in a neutral medium, was utilized in experiments given in Table 3. As may be seen, hydrolysate, prepared according to the first method, is not suitable for the development of the *Rhizobium*. It proved that the development of the alfalfa and soya bacteria in a soya or bean decoction begins earlier than in synthetic media; however, after a longer period of time, about 12 days, this difference gets smoothed, and synthetic media, may be, should be assigned the first place. It should be noted, besides, that nodule bacteria undergo in synthetic media in the course of time lesser modifications than in seed decoctions. In the latter the modification of the normal form of bacteria is earlier manifested, as well as granularity and even downbreak of cells. Bacteria are of a smaller size in synthetic media and preserve their rodlike shape. Generally the counting up of root nodule bacteria in a liquid medium has shown their development to be of insufficient intensity in either of the applied media. Best results have been obtained with a yeast hydrolysate, under condition of conducting hydrolysis in a neutral medium (Table 3), two details being extremely curious; firstly, hydrolysate gives the best results at a high, 70-fold dilution, which makes this medium economically extremely profitable. A considerably weaker development than in a 70-fold dilution is to be observed if the hydrolysate is being diluted 25-fold; yet a too far going 140-fold dilution already does not allow a good development of the bacteria. Secondly, the introduction of cane-sugar does not contribute to the growth of root nodule bacteria; in some cases it does not exert any influence, in others it markedly retards the development of bacteria in a liquid medium.

The above data show the yeast hydrolysate to be of a greater advantage, as compared to generally applied media; at the same time bacteria do not develop in it either with the intensity that should be desired. One of our next problems, the solution of which is perfectly necessary for preparing nitragin of a high efficiency, should be that of finding a medium, much more favourable for the development of the nodule bacteria than those that have been applied up to the present time.

As mentioned above, two ways may be applied at the research of methods for preparing effective nitragin; besides the first one — i. e. attempts made to find favourable conditions for

the development of bacteria in liquid media, the second one too was tested — the establishing of conditions most favourable for nodule bacteria to develop in the very preparations of nitragin. Were it possible to secure a strong multiplication of nodule bacteria in the preparation itself, namely in a sterilized soil, the problem of introducing into that preparation an emulsion of bacteria of the greatest possible concentration, would lose its acuteness.

The following problems were brought forward in the research of conditions, favourable to the multiplication of nodule bacteria in sterilized soil: 1) what is the type of soil the most favourable for the development of bacteria, 2) what is the % of moisture optimal for the multiplication of bacteria in sterilized soil, and 3) what are the chemical substances necessary to be added to a sterilized soil for obtaining the best development?

Experiments were run for the solving of the said problems, with sandy and peaty sterilized soils, with moisture varying from 50 to 100% of a full water-capacity, some — without introducing any foreign substances, others — with the introduction of CaCO_3 and of mannitol. Sterilized soils were inoculated with the emulsion of nodule bacteria, prepared by diluting cultures grown on solid media, the emulsion containing a number of bacteria, counted after the direct method of calculation. The test was invariably controlled by way of siftings and microscopic observations for making sure of the absence of any foreign contamination. The course of the development of bacteria was determined by means of the counting up of their quantity per 1 g. of soil after Winogradsky's somewhat simplified method; samples were taken with definite intervals of time, moisture was kept constant during the whole of the experiment.

Table 4

*Inoculation through nodule bacteria. 7.8 millions per 1 g. of soil.
Quantity of nodule bacteria per 1 g. of sandy soil (in millions).*

Series of tests	Days of the taking of tests	100%	50%	100%	50%
		of moisture		of moisture	
I. Sandy soils	15	36	24	27	31
	37	63	45	27	54
	75	12	10	8	6
II. Sandy soils with chalk	15	101	162	167	182
	37	180	299	188	380
	75	539	243	309	329
III. Sandy soils with chalk and mannitol	15	318	871	208	313
	37	775	149	708	1029
	75	734	696	795	710
Control	Microbes were not detected; in some cases only few were found in a quantity less than 1 million per 1 g. of soil				

Table 5

Inoculation with nodule bacteria. 3.9 millions per 1 g. of soil
Quantity of nodule bacteria per 1 g. of peat soil

Series of tests	Day of the taking of tests	100%	50%	100%	50%
		of moisture		of moisture	
I. Peat soil	15	8	3	2	2
	37	2	2	2	2
	75	1	0	0	0
II. Peat soil with 3 g. of chalk	15	163	570	117	226
	37	992	940	1 122	846
	75	746	841	962	1 425
III. Peat soil with chalk and mannitol	15	1 509	1 400	1 395	1 004
	37	2 193	1 627	1 838	1 717
	75	2 225	1 554	1 908	2 127
Control	Microbes not detected				

Experiments shown in Tables 4 and 5 make it possible to arrive at the following conclusions: nodule bacteria, inoculated into sterilized sandy and peaty soils at 50%—100% of full water-capacity without addition of chalk, do not multiply but regularly die off, which is manifested by an absence of growth after inoculation to nutritive media, as well as by a direct microscopical calculation; yet a multiplication of nodule bacteria is observed to take place in both soils under the same conditions, but at the entering of 3% of CaCO_3 ; this multiplication lasts in certain cases for 37 days, in others still longer. The addition to soil, besides chalk, of 0.6 of mannitol causes multiplication to be still more intense; corresponding to this, inoculations in a specifically suitable to the nodule bacteria Ashby medium give a more luxurious growth. The multiplication of nodule bacteria takes its course more energetically in a peat soil with chalk, than in a sandy soil; on the contrary, chalk being absent there takes place in a peat soil a still more rapid disappearance of bacteria. The increase of moisture from 50% to 100% of full water-capacity is not advantageous in the sense of the bacteria multiplication which is rather more rapid at 50% of moisture.

The data adduced show that, under condition of the soil applied for preparing nitragin being sterile, Winogradsky's method of direct calculation may be applied for a comparative counting up of nodule bacteria, contained in preparation in 1 g. of soil. The application of direct counting shows that one may reach by creating favourable conditions for the development of nodule bacteria in soil, their considerable propagation in

the very preparations: the maximum of propagation is to be observed after 37 days.

Activity of different strains

For elucidating the problem of the activity of different strains of the nodule bacteria, the necessity arises, first of all, of establishing methods for determining which of them are most active.

Data obtained by M. Löhnis and other authors give evidence that not all of the bacteria, able to form nodules, possess at the same time the capacity of fixing free nitrogen.

Therefore, when elucidating the problem of the activity of strains, one has to dwell upon the quantity of nodules, formed under the influence of inoculation, as well as on the capacity of individual strains to fix free nitrogen.

Our attempt to approach nearer the problem of the different ability of individual strains to fix free nitrogen upon sterile cultures of leguminous plants under laboratory conditions has not given any positive results.

The following deductions have been drawn relatively to methods for conducting experiments with inoculation of leguminous plants in sterile agar cultures: 1) the method of sterilization of each sort of seeds of legume plants requires to be individually checked. In some cases as in that, for instance, of seeds of lupine being infected not only over the surface but also underneath the pellicle, a more complex sterilization should be applied with the aid of 1% of bromine.

In respect to other seeds sterilization might be applied by means of strong hydrochloric acid during 15—25 minutes, and, finally, good results are obtained relatively to certain seeds, e. g. — clover and beans, by treating them with 1% solution of sublimate for 20—30 min.; the two latter methods are, comparatively, very simple.

The checking of the activity of nodule bacteria cultures was carried out in test-tubes with agar and the following nutritive medium:

Per 1 litre of water: NH_4NO_3 —0.24; $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ —0.172; CaCl_2 —0.15; MgSO_4 —0.06; $\text{CaCO}_3 \cdot 2\text{H}_2\text{O}$ —0.334; Fe_2Cl_6 —0.025, 1% of agar or 10% of gelatine.

The method of sterile cultures upon agar is suitable for the testing whether the strain being tested does produce the formation of nodules on the given legumen or not. This method, however, is not fit for elucidating which of the cultures are more active and which are less. At growth in test-tubes, a whole series of conditions do not allow the plant to develop normally. These various conditions may differently affect individual cultures and inhibit the obtaining of precise results relatively to the activity of different strains.

One may apply for elucidating the activity of separate strains only carefully conducted pot-culture or field experiments, taking into account the kind of the leguminous plant, as well as the peculiarities of the soil. One may, it seems, succeed in establishing, by means of that method, which are the most active strains for infecting a definite species of the leguminous plants.

Participation of different stages of the nodule bacteria development in nitrogen accumulation

The problem of the participation of different stages of development of the nodule bacteria in nitrogen accumulation in nodules remains, up to the present time, open to question; neither is elucidated the problem as to how takes place the enriching of leguminous plants with nitrogen, accumulated by bacteria in nodules. Two suppositions may be admitted in that respect: either bacteria are capable of secreting into the surrounding medium products of the nitrogen metabolism, which are further absorbed by leguminous plants, or bacteria, while in live state, preserve in their cell all the nitrogen assimilated, in the form of protein compounds. In the latter case only those of the nitrous compounds may be disposed of by leguminous plants, which are formed after the breakdown of bacteria cells.

For the purpose of elucidating problems connected with the participation of different stages of the nodule bacteria development in the fixation of nitrogen, experiments were conducted with lupine under field conditions, in which parallelly to microscopical observations on the course of the growth of nodule bacteria, the nitrogen balance of leguminous plants was determined.

Experiments were carried out, on a newly tilled poor sandy soil, which gave a very feeble natural contamination, for observing the accumulation of protein in lupine, in its relation to the stage of the development of nodule bacteria; the experiments, without inoculation being ended, nodules were found to be formed but in 15% of plants. The experiment was discontinued at the moment of the beginning of bean formation, i. e. before the drying up or loss of leaves which made it possible to count the average quantity of nitrogen per one plant of lupine. In order that an entirely precise picture might be obtained, samples were taken five times for microscopical observation, for the counting up of the increment of the green mass and that of the root system, for determining the quantity and weight of nodules, the percentage of nitrogen in separate organs of plants, as well as that of the infection of plants from inoculated and non-inoculated plots.

Inoculation was produced by way of infecting the seeds, and resulted in a 100% contamination, as well as in a vigorous

growth of nodules upon plants. Samples were mostly taken every three weeks; observations were carried out on the course of the development of nodule bacteria and on the determination of the content of nitrogen in lupine. The form of bacteria was studied in fresh nodules, as well as in fixed material; preparations stained with methylene blue and those not dyed were under observation. The first test for microscopical examination was taken at the very beginning of the formation of nodules, as soon as they had begun to get noticeable; in a week's time, after plants had begun being developed, nodule bacteria presented thick, slightly curved rods with obtuse ends and an even, granular content, staining easily. Bacteroides were already to be met at that early stage of the nodules' development and were, as usual,

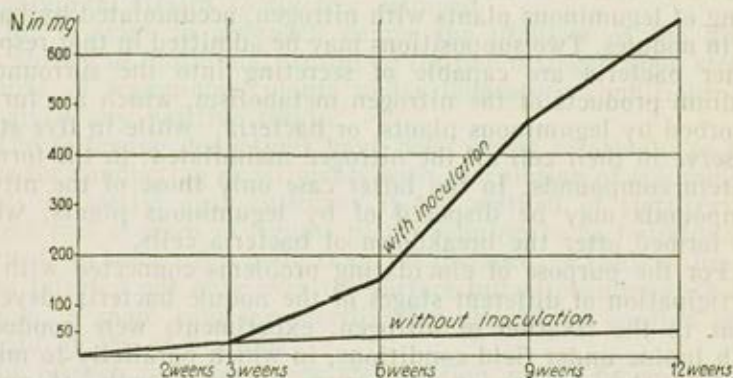


Fig. 1.

forkshaped. The second test was taken three weeks after the plants had begun growing, at the stage of 8—10 leaves; more definite still modifications in the shape of bacteria were to be observed at that moment. Shapes appeared of almost double the length, rods were curved much stronger, though staining easily. The third test, taken in a six weeks' time after the beginning of the plants' growth, corresponding to the formation on plants of 30—31 leaves, showed a considerable development of nodules. Nodule bacteria underwent at that period still greater modifications. No bacteria had remained unaltered; bacteria were either curved, or strongly, twice or thrice, lengthened, or forklike shaped.

A lengthened shape is one characteristic of the modification of the nodule bacteria of the lupine and is prevalent over all other forms. The content of cells of larger nodules begins differentiating into separate granular bodies, not so easily staining. The fourth sample was taken 9 weeks after the plant had begun developing and corresponded to the blooming of lupine

and to a complete development of nodules. The shape of bacteria in them was much altered; they were curved, twisted, often looking like spirals and being sometimes three or four times their normal length; very strong branchings were to be met. The content of bacterial cells was often highly granular. The fifth test, taken 12 weeks after the plant had begun developing, corresponded to the formation of beans; not only the shape of bacteria in nodules was found to be strongly altered, but a strong differentiation was noted in the content of bacterial cells, as well as a coarse granularity easily and strongly staining. A phenomenon is possible to occur at that stage, not observed at an earlier period and having to be subjected to detailed investigation, i. e. a dropping out or expelling of granular bodies from cells of bacteria.

Thus, the microscopical research gives a clear picture of a gradual rise of alterations in shapes of bacteria, of the conversion of bacteria into forms of bacteroides, proper to lupine. The fact is to be noted, being of special importance, that these forms of bacteroides undergo, in their turn, constant modifications in shape, as well as in content.

During the first three weeks of the experiment at the end of which no more than 6—8 leaves appear on plants, nodules only begin to get formed, the raw-weight of these on infected plants not exceeding 0.07 g. The most intense increase of the mass of nodules takes place, in infected plants, during the period of time between the 2nd and 3rd test, i. e. between the third and sixth week from the beginning of growth; further on this increase continues much more slowly, so that in a 12 weeks' time the mass of nodules surpasses that which had been formed in six weeks only by 26%. Another picture is to be seen in non-infected plants: whilst the chief mass of nodules has had time to be formed in a six weeks' period in infected plants, in those non-infected they only start growing that time, i. e. growth is considerably retarded. In connection with this, further increase in the mass of nodules is observed to take its course with equal intensity up to the end of the experiment.

During the first three weeks of the plants' growth no difference may be remarked in the weight of the green mass and the root systems of contaminated and non-contaminated plants, this being evidently, connected with a feeble development of nodules. Yet, further on, this difference begins to get strongly pronounced augmenting at the period between the 3rd and 4th tests before the blooming of plants, remaining after it, at the blooming period, almost unchanged. Hence, the period preceding blooming, between the 3rd and 4th tests, is that of the development of a leguminous plant, during which infection tells with peculiar acuteness on the difference in increment of the underground and overground mass of plant and during which the activity of the nodule bacteria in accumulating nitrogen is of a peculiar intensity. As to the

Table 6

Average weight of the green mass of roots and of the nodules of one plant of the blue lupine, one inoculated, the other not.

Conditions of experiment	I After 2 weeks, 4 leaves	II After 3 weeks, 6-8 leaves	III After 6 weeks, 32 leaves of contam. plant	IV After 9 weeks, blooming of contam. plant	V After 12 weeks. General blooming and formation of beans in contam. lupine
Per cent of plants					
Non-contaminated	6	8.6	18.6	14.6	14.6
Contaminated	82	89	97.2	99.3	100
Weight of the green mass of 1 plant					
Non-contaminated	1.50	2.14	6.7	11.4	18
Contaminated	2.50	2.42	16.9	60.3	104.6
Weight of the roots of 1 plant					
Non-contaminated	0.67	0.80	1.2	2.3	6
Contaminated	0.75	0.87	3.3	10.3	15.3
Weight of nodules of 1 plant					
Non-contaminated	—	—	0.024	0.29	0.56
Contaminated	—	0.07	0.192	2.03	2.60
Per cent of the nitrogen content in leaves					
Non-contaminated	3.77	2.93	2.32	1.81	1.65
Contaminated	3.28	3.12	4.35	4.80	3.66
Per cent of the nitrogen content in stems					
Non-contaminated	—	—	0.83	0.76	0.57
Contaminated	—	—	1.67	1.66	1.2
Per cent of the nitrogen content in roots					
Non-contaminated	1.23	1.25	0.83	0.69	0.72
Contaminated	2.44	2.52	2.87	2.24	1.97
Amount of total nitrogen in mg. per 1 plant					
Non-contaminated	5	15	36	46	50
Contaminated	6	14	136	471	664
Ratio of the amount of nitrogen in contamin. plants to that in plants non-contam.	1	1	4	10	13

course of growth of the green mass and root system, a definite difference has been noted between inoculated and non-inoculated plants, connected with a tardier development of nodules in the latter: beginning with the sixth week a rather equal increase of the green mass is evident in inoculated plants during the next six weeks; whereas in those non-inoculated this increase is speedier in rising during the last period due to tardy infection.

Seemingly, nitrogen fixation by nodule bacteria begins in the earliest period of their growth, when nodules cannot be perceived as yet or have just begun forming: in the root-system of contaminated plants a rise of the nitrogen percentage begins to be noted as early as two weeks after the beginning of growth, as compared to those non-contaminated; as to leaves, a rise in the nitrogen percentage of contaminated plants even, if observed, is very insignificant at that period of time. Three weeks after the beginning of growth the percentage of nitrogen begins to decrease, both in the green mass and in the root system of non-contaminated plants; in those contaminated, on the contrary, just three weeks after growth has begun, a marked rise in the percentage of nitrogen occurs in leaves; between the second and third test this rise is still more considerable; the percentage of nitrogen continues increasing, but a lesser intensity during the next three weeks, whilst commencing with the fourth test, i. e. after 6 weeks, it begins to decrease. The same course of changes in the percentage of nitrogen has been noted in stems. The maximum in roots is to be observed after 6, but not after 9 weeks, so that the decrease of the nitrogen percentage, seemingly, begins earlier. The determination of the dry mass of plants and of the percentage in them of nitrogen has made it possible to calculate the average of nitrogen per one plant, at different periods of its development.

As shown in Table 6 and in the curve (Fig. 1) differences in the amount of nitrogen in contaminated and non-contaminated plants were hardly to be observed in the first three weeks; this difference, i. e. increase of nitrogen at the expense of nodule bacteria, begins definitely to be observed 3 weeks after the beginning of the growth of plant. Increase of nitrogen in the plant of lupine, at the expense of the nodule bacteria, is equal to 100 mg. between the 2nd and the 3rd tests, to 325 mg. between the 3rd and 4th tests and, finally, to 185 mg. between the 4th and 5th tests. We may conclude from this, that the greatest energy of nitrogen accumulation is observed before the blooming of lupine, and that an energetic fixation of nitrogen by the nodule bacteria, as well as a transfer and adsorption of this nitrogen by leguminous plants, is taking place at that moment. The following conclusions may be arrived at, at the comparison of the course of development of nodule bacteria in nodules with that of the nitrogen accumulation in plants. Modified shapes of bacteria may be ob-

served in the earliest stages of the growth of nodules, at the time of their being hardly noticeable, as forked bacteroides of the usual shape are to be met in them, besides rods. One cannot judge of whether adsorption of nitrogen occurs thereby, as in a week's time after the beginning of growth only microscopical observations had been performed without chemical analysis. A chemical analysis carried out about a week later showed the percentage of nitrogen, two weeks after the beginning of growth, to be higher in the root system of inoculated plants, than in those non-inoculated. Three weeks after the beginning of growth microscopic observations were made, as well as an analysis for nitrogen. Further modifications in the shape of bacteria occurred at that period, in the sense of their lengthening and curvature; to judge by the capacity of bacteria for staining, no modifications in the content of cells had been observed at that time.

As recorded by data on the percentage of nitrogen in roots, bacteria begin fixing it at that early stage: the nitrogen percentage in roots of contaminated plants is almost the double of that contained in roots of those non-contaminated; nitrogen having not yet shifted at that period towards overground parts of plants, no difference is to be observed in the content of nitrogen in the overground part of inoculated and non-inoculated plants and in the weight of the green mass. During the next three weeks the shape of bacteria transforms with considerable intensity, and almost all bacteria have a modified shape by the moment of taking the third test. A considerable accumulation of nitrogen is to be noted during that period of time. The latter takes place with a maximum of intensity between the 3rd and 4th experiments, before blooming; almost all bacteria being highly modified, a differentiation of the content is to be observed, likewise a resistance to staining. At the next period connected with still greater alterations in the shape of bacteria, a rather intense, though less considerable, fixation of nitrogen by the nodule bacteria is to be noted. In connection with all the made observations one arrives at the conclusion, that the bacteroides, i. e. modified forms of bacteria, participate in the process of nitrogen fixation; bacteroides appear in the first stages of the growth of nodules, and the maximum of nitrogen accumulation occurs from the moment when all bacteria have modified shape and content. Further on, between the 4th and 5th experiments, accumulation of nitrogen is still to be noted, together with the shapes of bacteria undergoing still greater alterations. The fact that nitrogen accumulation at the expense of nodule bacteria begins in the earliest stages of the growth of nodules, and that this accumulation takes place with considerable intensity at periods when neither nodules nor separate cells of bacteria are observed to break down, makes one consider with discretion the possibility of the enriching of leguminous plants with nitrogen

being a result of the downbreak of bacteria cells; one should rather suppose that this enrichment takes place at the expense of the entering of products of the life-activity of bacteria diffusing from cells having a modified original shape, proper to bacteroides of lupine.

The participation of bacteroides in the fixation of nitrogen has been confirmed by other tests with lupine, conducted on podzolised loams, having higher supplies of nitrogen than sands, just spoken about. The difference observed, in these experiments in the increment of the green mass of plants is indicative of the influence, exerted by contamination, on the percentage of nitrogen in leguminous plants. During the first three weeks, beginning with the moment of the sprouting of lupine, no difference was to be observed in the green mass of contaminated and non-contaminated plants, nitrogen of soil fully satisfying the need of plants; further on supplies entering from soil could not entirely satisfy that need, and five weeks after the plant had germinated, just before blooming, the difference between contaminated and non-contaminated plants was manifested quite definitely: 13 g. and 8.5 g.; towards that time the greatest part of bacteria in nodules had their shape altered. These modifications in shape, lengthening of rods, twisting, brittleness and other alterations increase towards the time of taking the third test, 8 weeks' time after the beginning of germination, at the period of beans being formed, there takes place, simultaneously, an alteration in the content, and granularity appears. A much more energetic increment of the green mass of contaminated plants (from 13 g. to 58 g.), than of those non-contaminated (8.5 g. — 33.5 g.) occurs between the 2nd and 3rd tests which indicates an energetic assimilation of nitrogen by nodules of contaminated plants. This energetic assimilation coincides with the moment when most of bacteria have taken the form of bacteroides; thus, in that case too the participation of bacteroides in the fixation of nitrogen seems to be beyond doubt.

Influence of the character of soil on the activity of the nodule bacteria in leguminous plants

The question of the importance of inoculation in agriculture is closely connected with that of in how much the character of the soil, its richness in nitrous compounds and its physio-chemical properties affect the activity of nodule bacteria in nodules of leguminous plants.

It is not to be doubted that interrelations between nodule bacteria and the host-plant are extremely complex and, as yet, hardly elucidated. Even the problem of principle of whether it is symbiosis or parasitism that takes place in the given case, is differently solved by different authors. One of the problems

connected with it and being of great importance is that in how far the faculty of nodule bacteria for fixing nitrogen is controlled by the host-plant. One of the separate problems of that so wide main problem, is the following: how much a leguminous plants nitrogen supply from soil affects the fixation of nitrogen by nodule bacteria. For the solving of the latter problem experiments were carried out with soya bean plants upon chernozem soils and with lupine upon sandy soils and podzolised loams. Difference in increase of the total weight, and, especially, of that of the green mass of contaminated and non-contaminated plants, as well as the difference in the accumulation of nitrogen by these and the others — were taken as a criterion of the energy of the nodule bacteria activity relatively to the fixation of nitrogen.

Besides, experiments were conducted in sandy cultures, with and without bringing in sources of nitrogen, which made it possible to make clear with the greatest clearness the way in which the presence of readily available nitrogen in soil affects the nodule bacteria in respect to the fixation of nitrogen.

Influence of inoculation on the soya-bean yield.
Those non-inoculated are assumed to be 100.

Table 7

Denomin. of the leguminous plant	Soil	Volume or weight of nodules	Green mass	Total quantity of grain
Soya-bean plant non-inoc.	Thick chernozem	0	100	100
» inoc.	» »	7.0 cc.	104	107
» non-inoc.	Leached chernozem	0	100	100
» inoc.	» »	7.1 cc.	159	130
Lupine non-inoc.	Sandy soil	0.3 g.	100	—
» inoc.	» »	2.6 g.	480	—
» non-inoc.	Podzolised loam	0	100	—
» inoc.	» »	1.9 g.	400	—

Data are recorded in Table 7 on field experiments concerning the influence of inoculation of soya-bean plants on a thick and a leached chernozem. As may be seen, in spite of both cases being strongly contaminated due to the application of concentrated preparations of nitragin — yet no increase in the green mass and in yield is to be observed in the thick chernozem; control plants entirely free of nodules, and those infected do not differ in any way, one from the other. Another picture is offered in the leached chernozem: inoculation calls forth in it a doubtless increase in yield of the green mass, as well as of grain.

Extremely interesting data have been obtained in relation to the influence of the quantity and total mass of nodules on the total weight of the overground mass and of the soya-pods.

Table 8

Dependence of the growth of soya-bean plants from the different volume of nodules in two soils. Calculated per 1 plant.

Field experiments for the testing on nitragins.

Cis-Caucasus thick chernozems. Armavir Exp. St.				Leached chernozems. Gorsky Exp. St. (Vladikavkas)			
Volume of nodules	Total weight of the over-ground mass	Weight of pods in g.	Quantity of pods in g.	Volume of nodules in cc.	Total weight of the over-ground part, in g.	Weight of pods in g.	Quantity of pods in g.
7.0	232	98	63	10.4	282	93	76
3.5	232	120	100	8.0	252	76	78
2.0	200	94	84	4.4	214	60	63
0.8	198	94	91	1.0	191	59	36
0	232	112	91	0	162	50	37

On a thick chernozem in which the leguminous plant is fully provided with nitrogen, the quantity of nodules does not tell, in what so ever degree, on yield; on a leached chernozem not being able to fully supply a leguminous plant with nitrogen, a definite dependence may be seen between the increase of the green mass of the leguminous plant and the quantity of nodules upon it. The greater the volume of nodules, the more energetic is the fixation of nitrogen and the higher proves to be the yield. Seemingly, inoculation does not tell on the increase of the green mass of soya-bean plants on thick chernozem; however, there may be observed a certain rise in the percentage of nitrogen.

Experiments run with lupine on a sandy soil and on podzoli-sed loam have shown that, thanks to both of these soils not being secured with nitrogen, an abundant formation of nodules

Table 9

Nitrogen percentage in strongly contaminated soya-bean plants in thick chernozems

Single plants under field conditions. Armavir Exp. St. 1931.

	Number of nodule per 1 plant	Percentage of nitrogen				
		Roots	Stems	Leaves	Pods	Grain
Plants in the control plots	0	0.48	0.59	2.3	0.8	6.3
Plants inoculated through nodule bacteria	80—150	0.75	0.98	2.6	1.0	7.3

Contaminated plants and those of control do not differ one from the other in other respects.

Table 10

Percentage of nitrogen in soya-bean seeds.
Armavir Exp. Station. 1932.

NN of tests	Dates of the taking of tests	Conditions of tests	Quantity of nodules per 1 plant	Percentage of nitrogen	Average
4	31/VIII	Non-contam.	none	5.9	—
—	31/VIII	Max. cont.	numerous	5.2	—
5	20/IX	Non-contam.	none	5.4	—
—	20/IX	" "	"	5.3	5.3
—	20/IX	" "	"	5.1	—
—	20/IX	Max. contam.	numerous	6.2	6
—	20/IX	" "	"	5.9	—
7	19/X	Non contam.	none	5.8	—
—	19/X	" "	"	4.9	5.5
—	19/X	" "	"	5.4	—
—	19/X	Max. contam.	numerous	6.2	—
—	19/X	" "	"	6.1	6.2
—	19/X	" "	"	6.1	—

Table 11

Percentage of nitrogen in seeds, pods and leaves of the soya-bean plant.
Experiments at the maximum of inoculation upon leached chernozems in Vladikavkas.

Conditions of test	Nitrogen percentage of grain	Nitrogen percentage in pods	Nitrogen percentage in leaves
Non-inoculated	5.33	0.95	1.71
Inoculated through nitragin of the Inst. for Microbiology	6.82	1.95	3.21

produces a rise of the green mass of plants by 400% on a podzolised loam and by 480% on a sandy soil. The influence of nitrogen, readily available to a leguminous plant, on the activity of the nodule bacteria of the lupine has found its bright expression in experiments with sterile sandy cultures in pot-culture vessels with and without the adding of sources of nitrogen.

Observations on the formation of nodules have shown the bringing in of ammonium nitrate into sandy cultures to retard the formation of nodules. Data of the first and second test concerning the nitrogen percentage in plants, as well as the total amount of nitrogen in them, having been compared, have shown that the presence of nitrogen tells on the activity of nodule bacteria in nodules of leguminous plants. In the first test plants contaminated without nitrogenous salts are characterized by a lower content of nitrogen, than those plants which could dispose of a readily available nitrogen: the nitrogen was as yet feebly fixed by nodule

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Table 12

Importance of the entering of ammonium nitrate for inoculated lupine

Time of taking the sample	Inoculation	NH ₄ NO ₃	Total N in mg. within the whole plant	% N of the green mass
28 days	—	—	—	1.9
	—	+	103	5.2
	+	—	—	2.6
	+	+	126	5.2
46 days after blooming	+	+	421	3.15
	+	—	513	3.3
	—	+	435	2.7
70 days	—	+	471	1.2
	+	—	1096	2.85
	+	+	579	2.45

bacteria, and the plants gave an increase of nitrogen only at the expense of the readily available nitrogen of ammonium salts. At the second test all plants possessed an equal percentage of nitrogen, independently on their being inoculated, or on their utilizing nitrogen brought in as salt into sand; inoculated plants may be somewhat ahead of the others. The fact is worth to be noted that the addition of nitrogen in tests with inoculated plants does not cause any rise in the accumulation of nitrogen. The result is the same that occurred in chernozem soils — inoculation does not produce any increased accumulation of nitrogen, so long as the plant utilizes that readily available. By the time of taking the third test the readily available nitrogen has already been utilized by the plant, and nodule bacteria display their activity, in the sense of the accumulation of nitrogen, with full relief.

It should be noted that the delay in the formation of nodules, called forth by the presence of nitrous salts, has told on greater accumulation of nitrogen by inoculated plants without available nitrogen salts as compared with those which had these at their disposal.

These facts may be differently explained: either nodule bacteria, readily available nitrogen being present, badly develop and fix nitrogen, or they fix it in usual doses but do not utilize soil nitrogen. The delay in the growth of nodules affected by nitrous salts makes one rather incline towards the first supposition. This has been confirmed by observations on the effect of ammonium salts in the course of the development of nodule bacteria in nodules in similar sandy pot-culture experiments with the pea.

Correlation between the quantity of rods and bacteroides in nodules of the pea.

First test.	{	Vessels without nitrogen	80—85% of bacteroides
30 days		» with »	100% of rods
Second test,	{	Vessels without nitrogen	100% of bacteroides
blooming		» with »	100% » »
50 days	{	Vessels without nitrogen	100% of coccishaped rods
Third test.		» with »	100% » » »
70 days	{	Vessels without nitrogen	100% of coccishaped rods
		» with »	100% » » »

At the taking of the first test, nodules upon contaminated plants proved to be very small, their quantity being equal on plants having received as well as those not having received nitrogen.

By the time of taking the second test (full bloom), nodules on plants, not having received nitrogen, developed rather well, were numerous and increased in volume, yet not attaining the size observed under natural soil conditions (Detskoie Selo), whereas in plants that had received nitrogen they were few, very small, as though not fully developed. The following data were obtained at the counting up of nodules (only large, well developed nodules were counted up in plants without nitrogenous salts): contaminated without addition of nitrous salts — in the average 212 nodules per 1 plant; contaminated with addition of nitrous salts — 51 nodules; weight of nodules in the average per 1 plant in g. contaminated, without nitrous salts — 0.86; contaminated, with nitrous salts — 0.14.

Thus, it is perfectly obvious that the presence of such readily available forms of nitrogen as $(\text{NH}_4)\text{NO}_3$ stunts the growth of nodules.

By the time of taking the 3rd test (full maturing of beans), nodules on plants having received nitrogen had remained in an undeveloped state. The quantity and size of nodules in plants not having received any nitrogen was not altered as compared with the second test, but their consistency had become softer, and a modified mass oozed out of some of them at pressing.

A microscopical analysis of the content of nodules showed already at the first test of plants not having received nitrogen that nodules chiefly contained bacteroides. Rods were found in much lesser numbers; bacteroides were still absent in nodules of plants having received nitrogen.

Nodules taken in a second test from plants, some having received nitrogen and others — not, contained solely bacteroides, solitary rods being found in preparations.

In nodules of the third test coccishaped forms of rods were detected in all plants; bacteroides were absent.

Thus it proved that the course of the development of bacteria inside nodules of the pea coincides with that of nodule bacteria clover and vetch described by T o r n t o n. Thus, rods that had called forth the formation of nodules convert, in the course

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of time, into bacteroides that fully disappear at the time of the fruit maturing. By that time, the decaying nodule is filled up only with small coccishaped forms. Experiments carried out under field conditions with the same material, showed, that the course of development of bacteria inside nodules of peas fully coincides with the same in pot-culture experiments.

The presence of nitrous compounds, easily assimilated by a leguminous plant, tells on the course of the development of bacteria in the latter, retarding it, and thereby retarding as well the formation of nodules. The above data thus indicate that only a detailed study of the complex— nodule bacteria, legume plant, soil— will make it possible to elucidate the conditions of the activity of the two former under natural conditions. Relatively to the study of this complex, the problem is to be set first of all, as to the controlling agents conditioning the dependence of growth and the course of nitrogen accumulation in nodule bacteria on the nitrogen regime of the leguminous plant.

SOIL MICROBIOLOGY IN THE UKRAINE

(A. I. ROKITZKAYA

Ukrainian Sov. Republic, Kiev.

The study of problems of soil microbiology in the Ukraine has been started by endeavours of the Member of the Academy N. G. K h o l o d n y — in Kiev, and Prof. V. V. Z a l e s s k y — in Kharkov.

The original methodical works of N. P. K h o l o d n y on soil microbiology have great prospects in the practical study of the diagnostics and condition of soil.

A wide systematical organization of scientific-research works on problems of soil microbiology in the Ukraine, has to be referred to the year 1929, there being established at this epoch in Kiev, on the initiative of the President to the All-Ukrainian Academy of Sciences, Member of the Academy D. K. Z a b o l o t n y, — first a laboratory, and later on a section of Soil Microbiology at the Institute of Microbiology in the name of D. K. Zabolotny.

The scientific research work of the Section of Soil Microbiology of the D. K. Zabolotny Institute has been based on the principle of the closest concordance of field excursions work with laboratory research and stationary observations on microbiological soil processes, as far as possible, in a limited number of stations.

Special attention is concentrated on methods for studying microbiological soil processes, according to conditions of Ukrainian soils, which is of an enormous practical importance for the diagnostics of local soils.

Soil microbes are studied relatively to their pleomorphism, to laws of the variability, dissociation, and cycles of development, which is in direct connection with the formation of races; laws of mutation (saltations) are studied, and of physiological peculiarities connected with them, as manifested under natural conditions.

Microbiological soil processes are studied in complex associations, according to the character of the soil, conditioning the course of soil microbial processes under natural conditions. An immediate calculation of microorganisms in soils is found to be obligatory, as being of a particularly great importance for dynamics of microbiological processes.

The aim of the scientific-research works of the Section of Soil Microbiology at the D. K. Zabolotny Institute in Kiev — is the solving of the problem of raising soil fertility, in connection with which the thematics embraces problems of the zones of the nitrogen and humus funds of the Ukrainian soils, as well as those of the interrelation between plant and microflora in soil of the zones of its root systems.

Hence presented is the list of scientific-research works of the Section of Soil Microbiology of the Zabolotny Institute — published and being prepared for print.

1. R o k i t z k a y a, A. I. «Microflora of the ground and the water in the mines of the Donbass (Donets basin) and a new species of the microorganism «*Pseudomona Caerulea*» (was reported at the Microbiological Section of the II International Congress of Soil Science in Moscow, 1930).

2. Rokitzkaya, A. I. «Soil microflora in zones of root systems of plants in the region of forest steppe and chernozem in the Ukraine». (Report made at the Microbiological Section of the II Intern. Congress of Soil Science in Moscow, 1930, being prepared for print).

3. Rokitzkaya, A. I. «Vital phases of the soil celluloso-deströying microbe *Spirochaeta* *Cytophaga* *Huntchinson* and *Clayton*, and its spread in soils of the Ukraine». «Pedology» № 3, 1933).

4. Mayevsky, M. M. «To the problem of finding methods for isolating *B. Radicola* immediately from soil, independently on the host-plant» (being prepared for print).

5. Mayevsky, M. M. and Beskaravaynaya, T. F. «To the problem of the adsorption of microbes by soils» (being prepared for print).

6. Guesima, N. A. «Microflora of the seeds of sugar beets» (being prepared for print. Reported at the Scientific Conference of the D. K. Zabolotny Institute).

Actually being studied:

1. Actenomyces of the Ukrainian soils and the ir rôle in the decomposition of cellulose in soils (Rokitzkaya, A. I. and Beskaravaynaya T. F.).

2. Races of the *Azotobacter* connected with cultivated plants (Rokitzkaya, A. I. and Ruzhetchkova, V. I.).

3. Biology of a new thermophyle celluloso-deströying microbe isolated from peat of the Ukraine (Rokitzkaya A. I. conjointly with the Institute for Peat in Kiev. Laboratory of Dr Bogopolsky, M. D.).

4. Morphology and physiology of *B. Mycoides* in chernozems of the Ukraine and its relation to processes of nitrification. (Guesima, N. A.).

5. Influence on yield of the inoculation of the activated *Azotobacter* into soils of zones of the root-system of *Atropa Belladonna*, and medicinal properties of that plant. (Rokitzkaya, A. I.).

6. Denitrification in chernozem soils of the Ukraine. (Belling, A. M.).

7. Protozoa (Amoebae) in Ukrainian soils (Ruzhetchkova, V. I.).

8. Quantitative variations of soil microbes on the background of different organic fertilizers in chernozem soils (Shmaly, T.).

Laboratory of Soil Microbiology of the Institute of Agronomical Soil Science and Chemization of Agriculture. The Laboratory has been carrying out its investigatory work since 1928. (At that time the Microbiological Laboratory was attached to the Central Agrochemical Laboratory). Actually the staff of its workers consists of the director of the laboratory (Levantovskaya, V. M.), a specialist, an assistant, and assistant chemist, and technical staff.

Works on soil microbiology are conducted according to general programmes — complex themes, as well as on problems of a purely methodical order. The actual importance of problems connected with soil fertility and rise of yield have brought forward, first in turn, the study of the following problems:

1. Study and control of microbiological processes in manure, at using of different means of its preservation.

2. Study of the mineralization of the nitrogen stock, of the decomposition of cellulose, as well as of the dynamics of P_2O_5 , and Ca^{++} — as affected by irrigatory waters in the fields of the Kiev Agro-Industrial Combine manured with sewage.

3. Biological method for determining the need soils have in lime and in P_2O_5 , with the assistance of the *Azotobacter* and *Aspergillaceae*.

4. Spread of the *Azotobacter* in soils of the Ukraine dependently on the physico-chemical properties of these soils.

5. Influence of tractor tillage on the activity of the *Azotobacter*.

6. Energy of ammonification, — process contributing to the transformation of the nitrous organic substance, faintly assimilated by plants, into mineral compounds of those readily assimilated, studied on the background of the accumulation of humus.

7. Denitrification in different soil types, its dependence on the quantity and character of the organic matter, and the study of conditions for lowering the loss of nitrogen connected with it.

8. Influence of microbes decomposing cellulose on the development and activity of the *Azotobacter*.

9. Elaboration and checking of microbiological methods for determining need soils have in mineral fertilizers.

Division of General Microbiology with Section of Soil Microbiology at the Institute for Sugar Industry in Kiev. (Organized by A. I. Rokitzkaya in 1930). In the years 1931 and 1932 a series of tests were run by the laboratory in Experimental-Research mechanized Beetroot-Collective Farms, with the purpose of elucidating the most favourable combining of conditions, of the water-, air-, and nutritive regimes of soil which might secure the best course to be taken by physico-chemical and biological processes, contributing as well to the obtaining of high yields of sugar-beet and of other farming cultures.

Thus, in 1931 the influence of structure on soil processes and yield, likewise — on the condition of the soil microflora — were studied in a leached medium chernozem (beetroot soviet-farm Verkhniatchke) and in slightly leached chernozem, — in plots, with an artificial distribution of the structure of aggregates, by way of sifting through sieves. The total quantity of bacteria and fungi, the presence of azotofixing, nitrifying, denitrifying and cellulose destroying bacteria, of aerobs and anaerobs were computed at these microbiological investigations. Results obtained have proved the dependence of the microflora on the structural state of soil, at the given soil and meteorological conditions of the year 1931.

The tendency got outlined 1) to increase, soon after the tests had been started, the total quantity of microorganisms on plots with structural fragments < 3 mm. 2) to increase the % of the *Azotobacter* in plots with large aggregates (5–8, and > 8 mm.) and to diminish in the same plots the quantity of *Clostridium*. No quantitative alterations were manifested in respect to other groups of bacteria.

In 1932 experiments were carried out in three spots: Khristinovka — leached medium chernozem, the Shevtchenko soviet farm — slightly leached ordinary chernozem, and the Batieva Gora — podzolised Northern chernozem. Besides physico-chemical processes in soil the condition of microflora was also studied dependently on: 1) the structural state of soil in plots with artificially distributed aggregates, at different backgrounds of fertilizers, and 2) in plots affected in a different degree by implements of tillage at the ploughing previous to sowing. Results obtained under conditions of 1932, distinguished by a raised moisture, show that, under soil conditions of Khristinovka, the quantitative composition of the microflora depends on the structural condition of soil; this may be clearly seen in the scheme of a stronger action produced on soil by tillage before sowing, where in plots, first pulverized and after this having got more compact, the quantity of nitrificators has been decreased, while that of anaerobic groups — has increased. The total quantity of bacteria upon pulverized plots has got smaller. Under conditions of the first scheme, — artificial distribution of structural fragments, the total quantity of bacteria has decreased in variants smaller than 5 mm., soon after the test has been started. This difference in the quantitative composition of the microflora, has been still more emphasized at the entering of fertilizers, especially on the background of superphosphate — ammonium sulfate. The same tendency may be noted in soils of the Shevtchenko soviet farm where test were carried out according to the same schemes, yet with the difference that in these soils, having a strongly pronounced structure under conditions of great moisture-content, results have not been so clearly expressed.

Under soil conditions of the Batieva Gora, according to the scheme of an artificial distribution of structural fragments, the dependence of microflora on the structural condition of soil has also been manifested, i. e. a decrease of the total quantity of bacteria taking place in a plot with the fraction

< 1 mm. and an increase of it in plots with the fraction < 3 mm.; separate groups behave as though being under conditions of the Verkhniatchka.

Works for studying so well the microflora at various structural conditions as biological processes taking their course in them, have been conducted too in the Laboratory of Agrophysics at the Sector of Mechanization of the Ukrainian Agricultural Scientific-Research Institute for Sugar Industry (K o r n e v a, N. P. with collaborators).

In 1931 a Laboratory of General Microbiology with three sections: one for soil microbiology, the other — for technology, and a third — for general theoretical problems, were established at the All-Union Institute for the Study of Makhorka (*Nicotiana rustica*) in Kiev. The organization and management of this Laboratory was entrusted to A. I. R o k i t z k a y a.

Works on soil microbiology were directed towards the elaboration and study of concrete problems, converted with those of the makhorka industry. The central problem was that of raising yield and of obtaining a healthy tobacco-plant, thematics of the section having included in the plan the study of problems connected with the diagnostics and condition of soils of tobacco plantations on the background of fertilizers and crops rotation; after this the microbial regime was studied in zones of the roots' systems of *Nicotiana Rustica* (makhorka), soil microbes being also studied as indicators of the need of soils in fertilizers.

Those of the works on soil microbiology of the Institute for the study of Makhorka are to be noted:

1. Influence on the technical plant *Nicotiana Rustica* (makhorka) of the inoculation of activated *Azotobacter* into soil of the root system zone (R o k i t z k a y a, A. I.).

2. Microflora of soils of the makhorka plantations of the Lohvitz and Perevolotchansk Stations (L e b e d e v a, O. P., B e l t i u k o v a, K. I., O r l o v a - S h a k h o v a, A. A.).

3. The *Azotobacter* as being indicative of the need soils under makhorka plantations have in fertilizers (Calcium and phosphorus). (N a l i v a i k o, A. I.).

Microbiological laboratory of the Odessa Filial of the Biological Institute, started in 1930. Director Prof. L. I. R u b e n t s c h i k. The microbial composition of sea and limans (firth) is studied in this laboratory, as well as the fields of Odessa irrigated with sewage waters, and soils of the Odessa Agrocombinat.

The first communication of Prof. L. I. R u b e n t s c h i k on the aerobic decomposition of cellulose in fields irrigated with sewage waters, connected with the humusformation, may be found in the Transactions of the Odessa Filial, № 1, 1932.

The laboratory for microbiology carries out, apart from scientific-experimental work, also that scientific-instructive, with the purpose of preparing cadres of specialists.

There should be noted microbiological researches, carried out in the Ukraine, on lowland peat in connection with its utilization for national economy. Work is conducted by the Microbiological laboratory of the Ukrainian Filial of the Central Institute for Peat, organized in the Ukrainian Institute for Peat in February 1930, in Kiev. Work, is carried out under the leadership of the microbiologist M. D. B o g o p o l s k y.

Observations and deductions based on the work of this Laboratory. Peat reflects one of the historical phases of the rotation of substances in nature, conditioned by the removeability (changeableness) of uninterruptedly following bioprocesses. Bacterial processes are of a determining importance in processes of humic peat-formations, decomposition of peat and its mineralization. One may note, at peat being analysed, a marked difference in the bioproperties of the high- and lowland peats: in the pH, in chemical composition, in botanical composition; vacillations in the peat properties have been observed at examining a peat layer according to depth. The quantitative spread of bacteria in a lowland peat layer, in its upper interlayers, may be expressed in milliards

1 g. of peat of natural moisture-content contains 3.3—3 milliards of bacteria; periodical vacillations (increase and decrease) in the quantity of bacteria are to be noted at the investigation of the depth of the layer, dependently on the degree of decomposition of the layer of lowland peat; however, there has been established a gradual decrease, together with depth, of the quantity of bacteria (for instance, 1 g. of peat of natural moisture-content, contains 280—740 millions of bacteria at 3 m. depth, in different spots. In lowland peats (especially in dried up ones great) activity has been manifested by bacteria: those putractive, decomposing cellulose (under aerobic and anaerobic conditions), nitrifiers, denitrifiers in the upper interlayer — *Azotobacter* and others. Bacteriological processes in peat depend on properties, characterizing this or other kind of peat; the humate colloidal complex of the lowland peat conditions, to a great extent, the degree of activity or tardiness of bacterial processes in peat; degradation of the peat colloids (factors being: aeration, drying up and moistening — in turn) conditioning changes in the properties of peat stimulates the subsequent development of bacterial processes. Investigations have established the dependence of processes of the selfwarming of the freezer peat in pikes (stacks) on ground of modifications taking place in the peat-substances under the influence of a series of physico-chemical factors and the activity of a series of groups of microorganisms, of which, according to data of the said investigation, bacteria of a butyric acid fermentation acquire peculiar importance. Complex investigations of lowland peat collieries (Prof. Roll, Bogopolsky, Bersheda, and others) have established the possibility of organizing, a highly-effective pisciculture in ponds of the mentioned collieries. Researches of the Agricultural Sector conjointly with the Microbiological and Chemical Laboratories have established the possibility of a wide utilization of the lowland peat as litters, etc.

Besides all the above-mentioned, a whole series of problems has been elaborated on microbiological investigations of the lowland peat, freezer peat and isoplite peat (micrologist Z. K. Guizhitzkaya). Microbiological investigations on separate problems have been carried out at a simultaneous research of the given problems by other sectors and laboratories of the Ukrainian Institute for Peat-Agrocultural, Geobotanical, Hydrological, Peat, Chemico-Technological Laboratories, and others), all works being planned by the Institute.