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The taxonomic composition of soil microorganisms in the ecosystems of southern chernozems of Northern Kazakhstan

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This article discusses the species composition of soil microorganisms in the ecosystems of the southern chernozem of Northern Kazakhstan. Microorganisms are an essential soil component. In fallow soil where there has been no cultivation for over 50 years they amount to over 35 million cells per gram of soil, while in arable ground this increases to 46.2 million cells. Actinomyces tend to dominate in the biotic communities of uncultivated land. In cultivated arable land bacteria assimilating mineral and organic nitrogen predominate - a fact which testifies to the activity of soil-biochemical processes leading to profound mineralization of the organic substances in the soil.

Keywords: Soil microorganisms, soil biota, soil fertility, tillage, nitrogen

Introduction

Soils that are involved in agricultural production are influenced by powerful anthropogenic factors modifying the soil biological activity, physicochemical properties, and ultimately fertility. The study of the nature of the impact on soil microflora facilitates the selection process techniques, helping to improve soil fertility and its properties, makes it possible to predict changes in the level of cultivation. The most sensitive indicators, responsive to changing water and air, heat, soil nutrient regimes are soil microorganisms. However, despite the obvious need to study the soil microbocenosis, this issue is given little attention. Determination of the direction and intensity of soil-biological processes under the influence of anthropogenic change will allow establishing the level of biological activity, in order to select human impacts that have a positive impact on soil fertility and crop yields.

Soil - a complex system providing inhabiting place to the bacteria. Activity of these organisms influence on the nature and intensity of the biological cycle of substances, scope and intensity of the primary fixation of nutrient elements - nitrogen atmosphere, the ability to cleanse the soil, etc. (Polyanskaya, Geydebreht, Chernov, Pochatkova, and Zvyagincev, 2005).

According to researchers (Zenova, Orleanskiy, and Omarova, 2005; Zenova, Lihacheva, and Smirnova 2004), the types of soils are characterized by a certain ratio of bacteria, fungi and actinomycetes. The relative abundance of bacteria in the black earth soils is greater than in dark brown and podzolic soils. In the composition of bacterial flora in the soils of dry steppes increases the number of spore-forming bacteria, with a predominance of *Bac. megaterium* and *Bac. mesentericus, Bac. idosus* in contrast to the soils of north dominated by *Bac. mycoides. Bac. addlomeratus, Bac. sereus*. This indicates the different nature of the decomposition of organic matter in soils of different types.

Many researchers (Makarov, 2007) found *Azotobacter* in the black soil zone. *Azotobacter* development is usually found in chernozems in the spring, during the wet season; while is hardly found in dryland soil (Karaguyshieva and Illyaletdinov, 1957).

Mishustin (1965) found that the various representatives of the soil microflora respond differently to changes in moisture and temperature factors during the growing season. Seasonal fluctuations are attributive to spore-forming bacteria, including *Azotobacter* and nitrifying bacteria. The number of bacteria decreases sharply at the soil withering. In studying the effect of cultivation on the microflora of dark chestnut soils in Akmola region, it was found that cultivation significantly increases the number of ammonifying bacteria. Among the spore-forming bacteria in cultivated soils the widespread ones are *Bac. idosus, Bac. mesentericus, Bac. megaterium,* whose numbers are slightly decreasing with soil depth (Chulakov, 1971). On the whole thickness of the soil increases the amount of nitrifying bacteria and nitrate supply.

One characteristic of the southern black soil carbonate is a high content of actinomycetes, which are adapted to the development of low soil moisture. A large number of this group of microorganisms in chernozems creates unique conditions for the transformation of organic substance and soil humification (Mishustin, 1984). Studies of the distribution of actinomycetes in soils of Kazakhstan found that the black soil is less enriched with actinomycetes than the brown soil. And cultivated soils have more actinomycetes than the virgin soils. The species composition of actinomycetes isolated from soils of Kazakhstan is very diverse. Dominating species are members of *Streptomyces*, *Aureus, Griseus, Helvolus, Albus, Chrysomallus* and *Fuscus*.

Soil microscopic fungi - an important part of the soil microbial complex. It was found in recent years that despite the lower number of fungi compared with bacteria and actinomycetes, the total biomass of fungal mycelium makes up to 90% of the total soil biota in the soddy-podzolic soil, and up to 50% in the black soil (Mishustin, 1974). Mushrooms have much faster growth rate than bacteria; they secrete extracellular hydrolytic enzymes and develop in the first stage of microbial succession (Chulakov, 1975). A number of soil fungi produce phytotoxins that suppress seed germination, plant growth and causing soil exhaustion. *Penicilliim divaricatum*, *P. fuscipes* and *Aspergillus ustus* dominate in the complex of typical chernozem micromycetes in the control. *R.ribrit, Fisariut solapi, Talaromyces flavis* (= P.verticulatum) are met frequently. All of these fungi are active producers of phytotoxins: *P. fupiculosut* and *P. rubrum* - produce rubratoxin, *A. ustus* - ustic acid, *F. solapi* - fusaric acid, *A. flavus* - patulin. Among the typical, rare and accidental species are also many pathogenic fungi: *A.wenti, A. alliaceis, A.niger, T.wortmanntii* (Novoselov, Novoselova, Zavalin, and Gordeeva, 2006).

According to Mishustin et al. (1961), microscopic fungi in the southern zone of calcareous chernozems occupy a smaller part than in the brown and podzolic soils. When comparing the microflora of dark and light chestnut soils it was revealed a high content of microscopic fungi in the dark chestnut soils. The number of members of the genus *Penicillium* reduces in the dark chestnut soils; however, they remain the dominant group among the fungi.

The relatively high level of mineralization processes in the dark chestnut soils leads to a decrease in the content of fungi of the genus *Mucor* fungi and yeast, whose development is related to the content of slightly decomposed organic substance in the soil. Along with a decrease in the number of *Penicillium, Mucor*, and yeast-like fungi in soils of dry steppes increases the number of representatives of the genera *Aspergillus, Fusarium* and *Trichoderma* (Margesina and Mitevab, 2010).

The genus *Fusarium* is relatively rich both in the black soil and dark chestnut soils, is found in large numbers of the cultivated soils compared to the virgin ones (Jay Shankar Singh, Vimal Chandra Pandey, and Singh 2011).

Due to the change and pollution of the biosphere, which is due to growing of anthropogenic impacts in ecosystems, the role of soil microorganisms as biological indicators has increased. It is therefore necessary to know the conditions influencing the soil microorganisms activities in natural ecosystems, to determine the changes with microorganisms in agro-ecosystems, and to identify ways of their regulation (Polyanskiy, Golovchenko, Polonskaya, Guzev, and Zvyagincev, 2004).

According to Artamonova (2002), soil fungi actively participate in the biological cycle in northern ecosystems, while bacteria and actinomycetes dominate in the south in the structure of microbial cenosis. The author also identifies particular species of microorganisms in the functioning of various ecosystems. Ecosystems with low over mineralization processes (sod-podzolic and podzolic soils in particular) has the dominant species involved in the decay of organic matter in the early stages (*Bac. cereus, Bac. Virgulus, Bac. Agglomeratus*). A more profound transformation of organic matter takes place with the participation of *Bac. idosus*, *Bac. mesentericus*, *Bac. subtilis*. *Bac. megatherium* reproduces well in ecosystems with high availability of soil nitrate nitrogen. An indicator of saline soils is the *Bac. gasifican*. *Bac. mesentericus niger* is dominated in conditions of extreme aridity of ecosystems (arid regions of the zone).

The feature of virgin chernozem is the presence of thick layer of sod. The 0-24cm layer of one hectare of virgin soil contains much organic matter as it is contained in the 80-100 tons of manure (Sazonov, Manucharova, Gorlenko, and Umarov, 2005). Approximately 75% of the organic substance contained in a small surface layer which represents a continuum of felt plexus roots and plant residues. This layer of organic substance, which has a very important potential implications for a harvest in future time, has however a negative effect on yield in the early development of virgin lands: along with a high-grade humus it contains a large amount of non-decomposed plant residues. Microflora, associated with the processing of humus (nitrifying bacteria, humate mineralizers, Cellulose aerobic bacteria), is poorly developed in virgin soils compared to cultivated soil.

Recently, the importance of soil biota has increased significantly. Not only due to its indispensable role in the formation of soil fertility. In conditions of technogenic pollution of the biosphere, including soil, the soil biota performs another important function - detoxification of various compounds present in the soil and affecting the environment and quality of agricultural products.

The available information on the species composition of soil microorganisms in Northern Kazakhstan chernozems was presented by researchers during the development of virgin and fallow lands (Polyanskaya et al., 2005). Information on the microbocenosis development in modern soil and climatic conditions under anthropogenic impact on soils is limited. The study of the structure of microbial cenosis, and especially the species composition of microorganisms provides insight into the current soil-forming processes and the state of ecosystems.

Materials and methods

Soil microbiological examinations were made to determine the relation of physiological associations of micro-organisms and microbiological soil characteristics. The size and structure of the complex of soil microorganisms was determined by seeding dilutions of soil suspensions on dense nutrient media. The number of bacteria that use organic form of nitrogen was counted in the meat-peptone agar (MPA); bacteria and actinomycetes, using the mineral source of nitrogen on starch-ammonia agar (SAA), filamentous fungi - on acidified agar, Czapek-Doxa. Aerobic cellulolytic microorganisms were detected in the Hutchinson medium, followed by differentiation on bacteria, fungi and actinomycetes.

Meat-peptone medium (g/liter of tap water) is made from beef broth with peptone agar with the addition of 20 g. The medium was sterilized at 1 atm. During 30 min and poured into Petri dishes to 20-25 ml.

The composition of starch - ammonia medium (g/l tap water): (NH_4) 2 SO4 -2.0; K2HRO4 - 1.0; $MgSO_4$ -1.0; NaCl -1.0; $CaCO_3$-3.0, soluble starch- 10.0, agar-agar, 20.0.

Czapek's medium (g/liter of tap water): glucose - 20.0; KNO_3-5.5; $MgSO_4$ - 0.5; NaCl - 1.0; K_2HPO_4 - 0.4; $ZnSO_4$ - 0.002; $FeSO_4$ - 0.002; agar - Agar-20.0. pH-7.0-7.2.

Wednesday Hutchinson (g/100 ml): KNO_3 - 2.5; K_2HRO_4 - 1.0; $MgSO_4$ - 0.3; $SaCl_2$ and NaCl - by 0.1; $FeCl_3$ - 0.01; tap water, pH 7.2-7.3. The medium was poured into test tubes for two thirds of their height in each tube was placed for 4-5 strips of filter paper measuring approximately 5h1sm.

The selection of soil samples was performed by an envelope method to a depth of topsoil (0-10, 10-20, 20-30 cm), all work was carried out in compliance with the maximum sterility (presence of protective clothing, wiping the knife and spatula with alcohol, the presence of sterile packages). The soil moisture in selected soil samples was determined by drying to a constant weight at 105 °C.

To isolate the bacteria assimilating organic forms of nitrogen (bacilli), planting material was sown in MPA from dilutions of $1:10^3$. The volume of inoculum - 0.02 ml. Sowing of the suspension were plated in fivefold replication. The sterile medium served as a control. Petri dishes were cultured at a temperature of 28-30 ° C for 5-6 days.

To determine the number of spore-forming bacteria, the matrix soil samples were plated in suspension in sterile distilled water at a dilution of $1:10^1$, $1:10^2$, $1:10^3$ heated in a water bath at 80 °C for 15 min. Sowing was performed on the surface of MPA medium. Cups with crops were incubated at a temperature (29 ± 1) ^{0}C for 3 days. At the end of incubation, the bacteria colonies were counted taking into account dilution.

To determine the number of bacteria and actinomycetes, assimilating mineral forms of nitrogen, planting was made on the SAA medium surface from dilutions of $1:10^3$ by 3 Petri dishes. Cups were kept at a temperature of 28-30 °C for 7-10 days. At the end of incubation, colonies of bacteria and actinomycetes were counted taking into account dilution.

The number of filamentous fungi was determined by surface seeding of the soil suspension on Czapek - Doxa medium from dilutions of $1:10^2$. Cups were kept at a temperature of 28-30 °C for 7-10 days. At the end of incubation colonies of filamentous fungi were counted taking into account dilution.

To identify and record the aerobic cellulolytic microorganisms, the seeding was carried out in culture tubes with Hutchinson medium with strips of filter paper from a $1:10^2$ dilution in fivefold replication. The volume of seeds - 1 mL for each tube. Sterile medium with filter paper served as a control. Inoculated flasks were kept at a temperature of 28-30 °C for 12-14 days.

Results and discussion

South has a high humus biogenesis, and in the cultivated soil it is higher than in the virgin. The greatest number of soil microorganisms are concentrated in the upper layers of both virgin and cultivated soil. Down the profile, the number of microorganisms decreased, due to the depletion of soil humus and the deterioration of aeration, water-physical and chemical properties of the soil subsurface. However, in the distribution profile of virgin soil and arable land, each group of microorganisms has its own characteristics.

The studied soils has widespread ammonifying bacteria assimilating organic (accounting for the MPA) and mineral (on SAA) forms of nitrogen. The relationship between these bacteria can serve as an indirect indicator of the intensity of mineralization-immobilization balance in the soil.

Active mineralization processes of organic compounds occur in the soils of the southern carbonate chernozem, as evidenced by the high number of bacteria in the medium with assimilating mineral nitrogen. The highest value of SAA/MPA bacteria ratio is characteristic to the layer of 0-10 cm (Table 1).

TABLE 1. CONTENTS OF MICROORGANISMS AND THEIR DISTRIBUTION IN THE PROFILE OF CARBONATE CHERNOZEM (AVERAGE FOR 2009-2011)

Soil condition	Layers of soil, cm	Moisture, %	Total number of microorganisms million	Number of bacteria released in the media, Mln. in a gram of soil			Actinomycetes, Mln.
				Meat - peptone agar (MPA)	Starch-ammonia agar (SAA)	Ratio of SAA / MPA	
Virgin soil	0-10	23.0	16.3	0.6	14.5	24.2	0.798
	10-20	21.0	8.1	0.6	6.9	11.5	0.338
	20-30	17.0	10.6	0.4	9.8	24.5	0.361
Tillage	0-10	15.0	24.6	1.5	21.8	14.5	0.897
	10-20	16.0	14.9	0.5	13.4	26.8	0.560
	20-30	16.0	6.7	0.6	5.7	9.5	0.290
The least significant difference		1.29	6.63	0.05	4.66		0.106

Aerobic saprophytic bacteria are mainly represented by non spore-formers from the *Pseudomonas* genus, including fluorescent and pigmented bacteria and mycobacteria. Pigment form of bacteria is somewhat broader spread in cultivated soil, the fluorescent one is often found in the upper soil layers (0-10 cm).

The bacterial cenosis includes also spore-forming bacteria, most of which, in the studied chernozems, remains active in the vegetative form (accounting for the MPA, unpasteurized seeding). The higher, as compared to virgin land, number of spore-forming bacteria in the treated soil indicates on more dynamic process of profound transformation of organic substance. Since it is known that the spore-forming bacteria predominate in the later stages of decomposition, metabolize stable organic compounds that are difficult for other microorganisms. Groups, well-assimilating mineral forms of nitrogen, dominate in the bacilli composition: *Bac. idosus, Bac. megaterium, Bac. mesentericus.*

The actinomycetes in the upper horizon average 20% of the total number of amylolytic community bacteria in the SAA, and 12-13% of the total number of microorganisms (bacteria, fungi, actinomycetes).

The physiological functions of actinomycetes are very wide. This large group of microbes plays an important role in soil-forming process, participating in the process of synthesis - the mineralization of humus, metabolizing along with the bacilli the complex and resistant organic compounds (Smirnov, Trufanov, and Chebykina, 2006) and humic acids (Nikitin, 2002). From top to bottom on the profile of the southern carbonate chernozem number of ray fungi is reduced, but more gradually, compared to bacteria. The difference in content of actinomycetes in the soil virgin and arable land is negligible. The qualitative composition of actinomycetes (by type and color of colonies of aerial mycelium) is quite diverse. The most frequently encountered actinomycetes with pink, gray, dark brown color of aerial mycelium. Soil fungi are an important link in the development of fresh vegetable and animal remains. As the most demanding of oxygen saprophytes, they are mainly concentrated in the upper layers. Compared with the content of bacteria and actinomycetes, the number of fungi decreases significantly with depth sharply. With the depth of the soil fungal growth and reproduction in the virgin soil is reduced, not only because of the deterioration of aeration, but also due to decrease in the required forms of organic substance; this process is slower in arable land.

The embryos of *Penicillium, Trichoderma, Mucor, Fusarium* prevail in the generic composition of fungal flora, species of the genera *Alternaria, Dematium, Cladosporium*, and other dark-colored mushrooms are found in less quantity.

Bacteria and actinomycetes are common in the southern carbonate chernozems, mainly in arable land. According to Zvjagincev scale (1976), these soils can be attributed to the rich by the quantity of bacteria. High concentrations of bacteria assimilating mineral forms of nitrogen, germs, indicate on the direction of biochemical processes to the deep mineralization of soil organic substance.

Conclusion

In recent years, it is actively studied the composition of soil microflora as a biomonitoring parameter in assessment of the anthropogenic impact on soil during cultivation, polluting with heavy metals, pesticides and other chemicals. Intensification of agriculture leads to faster cycling of nutrients in the soil, a significant loss of humus, increasing its toxicity. These negative trends are largely attributable to changes in the structure of the soil microbial complex.

The practice of soil and environmental research points to the need for two types of environmental standards: natural standard - corresponding to virgin, undisturbed soil and their inhabitants; and environmental standard that emerged from the persisting anthropogenic influences that led to irreversible changes in soil microbial community and soil (Jay Shankar Singh, Vimal Chandra Pandey, and Singh 2011).

Based on the conducted studies it has been established that the processes of decomposition of organic residues on the virgin soil occur weakly. It is evidenced by the low number of assimilating mineral nitrogen bacteria (36%), soil fungi and cellulolytic microorganisms. Actinomycetes dominate in virgin soil biocoenosis. The same pattern was revealed in the reservoir, where the inhibition of the bacterial community is caused by micromycetes accumulation.

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