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The Effect of Cotton Monoculture and Alfalfa Crop Rotation of on Soil Microbiological Properties

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Annotation: The article describes the microbiological state of the soil in a long-term field experiment of a cotton monoculture and cotton-alfalfa crop rotations for many years (since 1926) under conditions of typical gray soils in the central experimental farm of the Research Institute of Agrotechnologies for Breeding, Seed Growing and Cotton Cultivation (RIABSGCC). The soil samples were taken at the beginning of cotton growing (spring) before the start of the experiment and at the end of cotton growing (autumn) and the amount of ammonifiers, phosphorus-decomposing bacteria, oligonitrophils, nitrogen fixers, micromycetes, actinomycetes from the studied active agronomic groups of microorganisms was determined. According to the results of scientific studies, nitrogen-fixing microorganisms in the soil were not found either in samples taken at the beginning of the application period, in spring, or in samples taken at the end of the application period in autumn. Decomposing bacteria were found in all variants at the beginning of the season. However, it was increased by the end of the application period in the 1st variant (a cotton monoculture, 30 t/ha of manure + 25 kg/ha of R2O5 (since 1926)). In the variant 4, it remained low rate of mineral fertilizers (a cotton monoculture NRK 150:100:75 kg / ha (since 1926)), and in other cases it was not found, it was stated that the number of oligonitrophils reduced in all variants, similar data were noted in micromycetes.

Keywords: a cotton monoculture, cotton, crop rotation, soil, microorganisms, mineral fertilizers, manure, standard

1. Introduction

It is known that soil fertility directly depends on its agrophysical and agrochemical properties, the amount of humus in the soil layers, organic and nutrient substances contained in it, especially the number of various beneficial microorganisms in its composition, and their active biological properties. In addition, the different geographical location of crop areas, the use of agro technical measures, biological processes in the soil, the targeted selection of cultivated crops depending on the characteristics of the soil require the development of modern agricultural practices. Our republic is one of the world leaders in cotton cultivation. In recent years, the volume of exports to a number of countries has been increasing. It is one of the important issues to study the effect of using mineral fertilizers and manure at different rates for many years on changes in the amount of microorganisms in the soil. In the life of microorganisms, the active exchange of nutrients, especially carbon from the main elements, and its circulation in green plants, their blue masses and the products obtained from them are of great importance.

The distribution, variety, and biological activity of existing organic and inorganic substances in the soil, especially microorganisms, are very important for the normal growth, development and high yield of agricultural crops, especially cotton. Therefore, one of the most urgent tasks is to study the condition, quality,

and composition of the soil of cultivated fields in our country, to study chemical, biological, and especially microbiological processes in them, to improve soil structure, and to increase productivity. It is known that intensive technologies, including both mineral and partially organic (manure, green manure) fertilizers are used to obtain high yields of agricultural crops

The use of these fertilizers allows not only to obtain a high yield from agricultural crops and enrich its microflora, but also to improve the soil microstructure, increase plant resistance to various diseases (gommosis, fusarium, root rot, etc.) and improve the quality of the crop.

Life on Earth is supported by two main processes - the creation of new organic substances through photosynthesis and their subsequent gradual decomposition. The first is mainly carried out by higher plants, and the second by microorganisms in the soil. The formation and dynamics of the biochemical, nutrient, air regimes of the soil are closely related to the activity of microorganisms. All this indicates that microorganisms play a very important role in maintaining and increasing soil fertility [1, 10, 2, 4].

Microbiological monitoring includes the study of the abundance of the main agronomically important groups of soil microorganisms, the participation of nitrogen, phosphorus, potassium, carbon in the soil, as well as other trace elements - ammonifiers, oligonitrophils, phosphorus-mobilizing bacteria, actinomycetes and micromycetes. in the topsoil. In the soil, the strongest proteins, which make up 50% of the dry mass of the cell, break down faster. Proteins are decomposed by ammonifiers, actinomycetes, micromycetes (fungi). When proteins are broken down by microorganisms, nitrogen is released in the form of ammonia. This process is called ammonification. Ammonification is of great importance for plant nutrition. Oligonitrophilic bacteria have the ability to absorb nitrogen from the atmosphere and convert humus carbon into useful forms. Their accumulation in the soil can greatly contribute to nitrogen enrichment. In this regard, a comprehensive study of this group of microorganisms is of great importance for increasing soil fertility. Phosphormobilizing microorganisms convert mineral and organic phosphorus into a form convenient for absorption by plants, and micromycetes, also include phytopathogenic fungi that cause various diseases of agricultural crops. And actinomycetes fight diseases of agricultural crops [2, 3, 4].

Microorganisms play an important role in the formation of humus in the soil and in the conversion of chemicals needed by the plant into a state suitable for absorption by the plant. After all, the composition of the soil microflora and the metabolism of microorganisms interact with the mineral and organic parts of the soil [5].

R. O. Oripov [6] argued that the microbiological processes occurring in the soil throughout the year are the main phenogen in increasing soil fertility. Therefore, in order for microbiological processes in the soil to continue throughout the year, it is necessary to increase their species, abundance and biodiversity in crop rotation systems.

The same idea was confirmed by S.A. Vorobyov [7], and the abundance of crop species in the crop rotation provides the plant with the necessary nutrients. An important part of this process is that they undergo rapid microbiological decomposition; in many cases, it surpasses organic fertilizers in terms of the degree of humus formation.

According to F. V. Turchin [8], leguminous crops, which are sown alternately throughout the year, provide useful microflora in the soil. Depending on the enzymatic properties of microorganisms, nutrients that are difficult to assimilate by plants are also used.

To preserve and increase soil fertility, it is first necessary to improve the microbiological properties of the soil. The fertility and productive properties of the soil are closely related to the development and activity of soil microorganisms [9].

Bacteria and other microorganisms are more common in nature than other living organisms. Since they are extremely small, they consume different types of food, actively participate in the transformation of one substance into another, to be active in the acceleration of physical and chemical processes in the soil and water, at the same time it varies depending on the plant and animal residues in the soil, soil type, moisture and other characteristics. It is also affected by changes in soil properties [11, 15].

Also, in many cases, the activity of microorganisms is better in the fields using crop rotation compared to a monoculture cultivation [16], and with a monoculture care, a decrease in non-phytopathogenic fungi is observed. compared to phytopathogenic fungi [17]. Therefore, in Turkey, the government decided to prevent a monoculture cultivation of crops, and from 2020 they refused to sow the same variety of crops in the same field for more than three years [18].

In the Huanghe River Valley of China, when grown cotton and wheat together the yield in the first year was less compared to the cotton continuous crop field, but the soil fertility increased. The cotton yields further declined to 38.8% on marginal soils and to 22.7% on highly fertile soils. The economic performance has also increased compared to a cotton monoculture care [19].

In fact, if the agrophysical, water, water-physical properties of the soil are moderate, the movement of microorganisms in it is activated, as a result, soil fertility increases. Therefore, an important issue is to study the degree and scope of the influence of crop rotation on the activity of microorganisms in the soil.

2. Research methodology

The field experiments were carried out at the central experimental field of the Research Institute of Agrotechnologies for Breeding, Seed Growing and Cotton Cultivation of the Kibrai District of the Tashkent Region under conditions of typical gray soils. The central experimental site is located 7-8 km from the Chirchik River, on the right bank of the Boz-suv canal, in the north-eastern part of Tashkent, in the Kibray district of the Tashkent region. The Institute is located in the foothills, in the direction of the south-west wind from the slope of Mount Karjan, which is part of the Chotkal Range. The soil-parent rock is uneven, typical old-irrigated sierozem. According to the mechanical composition, the soil of the experimental field is heavy sand, groundwater is at a depth of 18-20 meters.
The studies were carried out in the field, under which land preparation, sowing, intercropping cultivation, watering, phenological observations of plants in the care of cotton were carried out on the basis of the generally accepted methodological manual "Methodology for Conducting Field Experiments"[19].
The methods generally accepted in soil microbiology were used for microbiological analysis of soil samples [2, 3, 4].
The soil samples were taken from a depth of 0-30 cm to study the amount of the main physiological groups in the soil. The studied soil and water microorganisms, including: ammonifying bacteria - GPA medium, oligonitrophils - Ashby medium, micromycetes and actinomycetes - Czapek's solid medium were studied. A suspension was prepared from a soil sample that taken for microbiological analysis. To do this, 10 g a sample of soil was taken, mixed with 90 ml of sterilized water and shaken for 5 minutes, then 1 ml of the suspension was taken with a pipette and placed in 9 ml of water in a sterilized test tube. This process was continued serially, diluted to 1:1000000 and repeated. 1 ml of liquid in a test tube was sown on special dense selective nutrient media in Petri dishes in triplicate, i.e. on the basis of "dilution" and examined.

№	The composition of the Ashby nutrient medium includes the following substances:	The composition of the Capek nutrient medium includes the following substances:
1.	Sucrose 20 g	Glucose- 20 g
2.	Potassium phosphate 0,2 g	Sodium nitrate 2,0 g
3.	Magnesium sulfate 0,2 g	Potassium hydrophosphate 1,0 g
4.	Sodium chloride 0,2 g	Magnesium sulfate + 7 water 0.5 g
5.	Potassium sulfate 0,1 g	Potassium chloride 0.5 g
6.	Calcium carbonate 0,5 g	Calcium carbonate 3 g
7.	Agar 20 g	Agar 20 g
8.	Water 1000 ml	Water- 1000 ml

The number of bacteria, actinomycetes, and fungi per 1 g of dry soil was calculated using the formula;

$$а = \frac{б \times в \times г}{д}$$

in that; а – the number of cells in 1 g of dry soil,
б– the average number of colonies in a Petri dish,
в – plot used for planting,
г – the number of drops in 1 ml of suspension,
д – weight of dry soil taken for analysis.
The total area of the field experiment is 1,6 ha, each variant is 2000 m^2 and includes:
Variant 1: cotton monoculture, 30 t\ha manure +25 kg\ha P2O5 (since 1926).
Variant 2: cotton monoculture NPK 250:175:125 kg\ha (since 1926).
Variant 3: cotton monoculture, no fertilizer (since 1926).
Variant 4: cotton monoculture NPK 150:100:75 kg\ha (since 1926).
Variant 5: Crop rotation 3:7, alfalfa:cotton NPK 150:100:50 kg\ha (since 1936).
Variant 6: Crop rotation 3:7, alfalfa:cotton NPK 150:100:50 kg\ha, manure applied at 30 t\ha in year 4 (since 1986).
Variant 7: Crop rotation 3:7, alfalfa:cotton, no fertilizer (since 1986).

Variant 8: Crop rotation 3:7, alfalfa:cotton, 10 t/ha of manure annually, (since 1986).

3. Results and discussion

The decomposition of organic matter occurs due to the biological uptake of organic matter in the soil by microorganisms. The soil is home to a wide variety of microorganisms: bacteria, actinomycetes, fungi, algae, yeasts, lichens and simple benthic animals. Their number is extremely variable, the number microorganisms in 1 gram of soil is millions and billions. reaches up to. Also, through the microbiological activity of the soil, its properties, regimes and fertility are formed. One of the important issues is the study of the microbiological activity of soils in order to understand the causes of soil processes, properties, modes and current conditions of productivity, assess productivity and manage it in the right direction.

In soils, proteins decompose most rapidly and account for 50% of the dry mass of cells. Proteins are broken down by ammonifiers- aerobic and anaerobic bacteria, actinomycetes and fungi. As a result of the breakdown of proteins by these microorganisms, nitrogen is released in the form of ammonia. The process of ammonification is of great importance in plant nutrition. Oligonitrophis plays an important role in the transformation of nitrogen and carbon in the soil. This group of microorganisms breaks down the carbon part of the most important organic matter. Nitrogen-fixing bacteria have the ability to absorb nitrogen from the atmosphere. Their accumulation in the soil can cause it to be enriched with a certain amount of nitrogen.

Actinomycetes are common soil microorganisms. Actinomycetes absorb organic and mineral forms of nitrogen, and the are able to decompose mono-, di-, and polysaccharides, as well as animal and vegetable oils. Some actinomycete are able to decompose soil humus and chitin. Actinomycetes are resistant to high salt concentrations, some of them are able to accumulate nitrogen in the atmosphere. Along with other soil microorganisms, the soil microscopic fungi play an important role in soil fertility. A large number of their species are actively involved in the decomposition of plant residues in the soil. The soil fungi play an important role not only in the biological processes occurring in the soil, but also in plant life. The significance of the flora of fungi in nature and in human economic activity is enormous. In particular, many medicinal substances, antibiotics, and enzymes are isolated from fungi, while they caused a number of diseases in animals and agricultural crops. Therefore, the study of soil fungi is not only of scientific and world significance, but also of great practical importance. The works of many scientists show that the soil of Uzbekistan contains significantly less fungi spores than the soils of the republics located in other soil-climatic regions. These data are also confirmed in our studies. This is due to the harsh soil and climatic conditions of this country - lack of moisture, alkaline reaction of the soil mixture, low organic content, very dense soil. The number of microscopic fungi depends on the degree of cultivation of the soil, its season.

Table 1The number of microorganisms of the main physiological group in soils, CFU/g of soil (CFU/g –column forming unit/ in gram soil) At the beginning of the spring

№ Samples	Types of microorganisms					
	Ammonifiers	Phosphorus – decomposing bacteria	Oligonitrophils	Nitrogen fixers	Micromycetes	Actinomycetes
1	$6,3x10^7$	$1,9x10^6$	$1,0x10^6$	was not found	$1,5x10^3$	was not found
2	$9x10^6$	$2,1x10^6$	$2,7x10^6$	was not found	$1,5x10^3$	was not found
3	$1,2x10^7$	$1,5x10^5$	$5,4x10^5$	was not found	was not found	was not found
4	$3,7x10^7$	$1,5x10^5$	$1,4x10^6$	was not found	was not found	was not found
5	$1,5x10^6$	$9x10^5$	$1,8x10^6$	was not found	$1,5x10^4$	was not found
6	$1,5x10^8$	$1,1x10^6$	$1,9x10^6$	was not found	was not found	was not found
7	$6x10^6$	$7,5x10^6$	$2,1x10^6$	was not found	$3x10^4$	was not found
8	$6x10^6$	$2,2x10^6$	$1,0x10^6$	was not found	$1,5x10^3$	$3x10^3$

As a result of the microbiological analysis, in the studied soil samples the number of ammonifiers bacteria ranged from 10^6 to 10^8 CFU cells per 1 g of soil. The number of ammonifying bacteria turned out to be higher in variant 6 compared to other samples and amounted to $1,5x10^8$ CFU cells/g. In variants 1, 3, and 4, their number was $1,2\text{-}6,3x10^7$ CFU cells/g. In variants 2,5,7 and 8, it was $1,5\text{-}9x10^6$ CFU cells/g. In variants 4 and 7, *Bacillus mycoides* was found from bacterial species belonging to the *Bacillus* and amounted to $1,5x10^5$ and $1,5x10^6$ CFU cells/g.

Phosphorus degrading bacteria were found in all analyzed samples and their number ranged from10^5 to 10^6 CFU cells/g per 1 g of soil. In variants 1,2,6,7 and 8, their number was $1,1\text{-}7,5x10^6$ CFU cells/ml. In the other variants, phosphorus-degrading bacteria were less common and accounted for $1,5\text{-}9x10^5$ CFU cells/g.

The number of oligonitrophilic microorganisms in the 3rd variant was an order of magnitude less than in the other variants, and amounted to $5,4x10^5$ CFU cells/g. In all other variants, their number was of the same order (10^6) and amounted to $1,0\text{-}2,7x10^6$ CFU cells/g. The studied soil samples were not only rich in micromycetes, but also contained different types of these microorganisms.

The soil samples analyzed for microscopic fungal counts showed 103 to 10^4 CFU cells per gram of soil. The presence of CFU cells was observed. In variants 5 and 7, their number was $1,5\text{-}7,5x10^4$ CFU cells/g. In variants 1, 2 and 8, their number was less than one order of magnitude and amounted to $1,5x10^3$ CFU cells/g. In variants 3,4 and 6, micromycetes were not found at all. The fungi of the genera *Mucor*, *Aspergillus*, *Penicillium* and *Fusarium* were found in the studied soil samples.

Actinomycetes were found only in variant 8 and their number was $1,5\text{-}3x10^3$ CFU cells/g. in other cases, they were not found at all.

In conclusion, as a result of studying the microflora of these studied soil samples, it was noticed that the number of ammonifying and phosphorus- decomposing bacteria from the main physiological group of microorganisms in all samplesis 1 and 2 orders of magnitude less than the norm, the number of oligonitrophic microorganisms was normal, and azotobacter and actinomycetes were less than normal or not detected at all. Micromycetes were normal only in variants 1,2 and 8 and in the remaining samples it was found an order of magnitude higher than the norm. There were various types of micromycetes. Species of *Bacillus mycoides* belonging to the genus *Bacillus proved to be dominant species.*

By the end of the season, it was noted that the main physiological group of microorganisms in the soil had changed in different ways. The results of microbiological analyzes have been presented in table. 2.

As a result of the microbiological analysis, the number of ammonifying bacteria in the studied samples ranged from 10^6 to 10^7 CFU cells per 1 g of soil. The number of ammonifying bacteria was $3x10^6$ in the 3 rd unfertilized control where cotton was grown for many years in the same field. When compared to the beginning of the season, it has decreased by one order of magnitude. Phosphorus- decomposing bacteria and actinomycetes were not found. The number of oligonitrophils somewhat decreased, and the number of micromycetes by the end of the season was $1,5x10^3$. In the variants 1, where 30 t/ha+25 k g P_20_5 fertilizer was applied, ammonifiers and phosphorus- decomposing bacteria decreased by an order of magnitude compared to the beginning of the season. It was found that oligonitrophils increased by an order of magnitude, while micromycetes did not change.

Phosphorus-decomposing bacteria were found on all variants at the beginning of the season, but by the end of the season they were found only in variants 1 and 4, and their number per 1 g of soil was $1,5\text{-}3x10^5$ CFU cells/ml in the samples. In the samples 9 and 10 it was $1,5\text{-}1,8x10^6$ CFU cells/ml. In the rest samples phosphorus- degrading bacteria was not found. The main function of nitrogen fixers is the absorption of free nitrogen from the air by plants. Nitrogen fixers were not found in the a cotton monoculture fields and cotton crop rotation. Therefore, the absence of this type of microorganisms could not be determined due to the constant care of cotton.

Table 2 The number of microorganisms of the main physiological group in soils, CFU/g of soil (At the end of the autumn)

Вар т/р	Types of microorganisms					
	Ammonifiers	Phosphorus – decomposing bacteria	Oligonitrophils	Nitrogen fixers	Micromycetes	Actinomycetes
1	$9x10^6$	$3x10^5$	$4,8x10^5$	was not found	$3x10^3$	was not found
2	$6x10^6$	was not found	$1,8x10^5$	was not found	was not found	was not found

3	$3x10^6$	was not found	$2,4x10^5$	was not found	$1,5x10^3$	was not found
4	$1,8x10^7$	$1,5x10^5$	$1,8x10^5$	was not found	$1,5x10^4$	was not found
5	$1,3x10^7$	was not found	$6,0x10^5$	was not found	$3x10^3$	was not found
6	$4,5x10^6$	was not found	$5,8x10^5$	was not found	$1,5x10^4$	was not found
7	$5,7x10^6$	was not found	$3,7x10^5$	was not found	$1,5x10^4$	was not found
8	$1,6x10^7$	was not found	$7,5x10^5$	was not found	was not found	was not found

It was established that the number of oligonitrophic microorganisms was in the same order (10^5 CFU cells/g) of the soil in all variants and amounted to 1,8-8,7x10^5 CFU cells/g. At the beginning of the season, only the control (without fertilizers) remained unchanged. In all other variants, it was found that by the end of the season it was decreased by one order of magnitude.
The soil samples analyzed for the number of microscopic fungi (micromicides) showed that 10^3 to 10^4 CFU cells were present in 1 g of soil. In variants 4, 6 and 7, their number was 1,5-4,5x10^4 CFU cell/g. In variants 1, 3 and 5, their number was an order of magnitude lower and amounted to 1,5-3x10^3 CFU cells/g. In variants 2 and 8, micromycetes were not found at all. The fungi belonging to the *Mucor, Aspergillus* and *Fusarium* were found in the studied soil samples. Notably, the variants 3, 4 and 6 were not found in the analyzes conducted at the beginning of the season.

4. Conclusion

In conclusion, as a result of studying the microflora of these studied soil samples, as a result of the constant content of cotton in one field, it was noted that the number of ammonifiers, phosphorus-decomposing bacteria and oligonitrophilic microorganisms from the main physiological group of microorganisms in all samples is less than the norm for soils, and nitrogen fixers and actinomycetes were not found at all. Micromycetes only in the variant 1, where monoculture cotton is sown 30 t/ha manure + 25 kg/ha P2O5 (since 1926) was applied. In the variant 1, a cotton monoculture, without fertilizers (since 1926), in the variant 3 and crop rotation 3:7 alfalfa: cotton NPK 150:100:50 kg\ha (since 1936) was applied. In the variant 5 turned out to be within the norm for the soil, and in the other variants it turned out to be an order of magnitude higher than the norm. In general, the main physiological group of microorganisms changes as a result of various applied agrotechnical measures.

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