The content of bacteria, yeasts and fungi in the soils of apple orchards in the north CCR

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Abstract. The aim of our research was to study the ratio of bacteria, yeast and fungi in soils occupied by apple orchards of different ages in the northern part of the Central Black Earth Region and fragmentarily in the Stavropol Territory. The research was carried out in 2015-2017 in fruit growing farms in the Tambov, Lipetsk, Penza regions and the Stavropol Territory. The objects of research were soils (typical chernozem, leached, podzolized, meadow-chernozem, chernozem-meadow, chernozem-moist-meadow and gray forest soils) under industrial apple orchards. The gardens were laid out according to 5x3, 6x4 and 6x8 m schemes on stocks 62-396, 54-118 and seed. The age of the surveyed gardens is from 15 to 89 years. In the waterlogged chernozem-meadow and chernozem-moist-meadow soils, the highest number of bacteria was noted, but the lowest number of fungi. The greatest amount of yeast and mold fungi (favorable for the apple rhizosphere micro flora) is noted in gray forest soils and podzolized chernozems. In the root layers of the near-stem strips of apple orchards, there were 2.3 times less bacteria, but 3.8 times more yeast and 4.4 times more fungi than in the aisles. There are more bacteria, yeast, and fungi in the humus horizon of the near-stem strips of old apple orchards than in the soils of younger orchards. As the age of the garden increases in the ratio "bacteria: yeast: fungi", the proportion of yeast increases 2 times, and the proportion of fungi - 1.5 times. A year after stubbing the gardens in the 0-40 cm soil layer, the amount of molds becomes average between their content in the near-trunk strips and aisles. The death of trees on chestnut soil was noted where there were 5 times more bacteria, 2.5 times less yeast and 2 times less fungi.

1. Introduction

When designing intensive gardens, one should know its microbiological properties [1-9]. As a result of the accumulation of plant polyphenols in soils, the structure of the microbial community changes and soil fatigue sets in [2-4]. In the near-stem strips, the presence of leaf litter determines the constant presence of cellulose-decomposing bacteria Enterobacter cloacae in the soil [3]. The use of herbicides in gardens strongly affects the genera of bacteria from the order Chthoniobacterales and reduces the...
number of antagonists that fight pathogens [11]. The presence of pathogenic bacteria in the soil (Klebsiella pneumonia, Shigella spp and E. Coil. etc.) indicates soil contamination [13]. The bacteria Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa and Escherichia coli in garden soils show resistance to a wide range of antibiotics and heavy metals such as zinc, copper and lead [6], and some bacteria (Lysinibacillus sp., Bacillldoco sp. And Rho.) even reduce the concentration of metals in the soil [5]. The bacteria of the genus Pseudomonas sp. strain HA-09 revealed a gene responsible for resistance to herbicides Glyphosate and Roundup [7]. Acidic soils have more bacteria, therefore fruit trees are more susceptible to diseases [10], but some bacteria (Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Nitrospira, and Proteobacteria) increase the availability of calcium and magnesium for apple roots [17]. The rate of nitrogen conversion depends on the ratio of bacteria and fungi in the soil under trees [12-13]. The ratio of bacteria and fungi and their species diversity depends on the soil maintenance system in the apple orchard [15-17] and on the doses of organic fertilizers [18]. Vineyard soils have a higher species diversity of fungi than in other fruit plantations [11]. The biological activity of the soil is directly dependent on its density [8]. The deterioration of the physicochemical and morphological properties of soils between rows of gardens leads to a decrease in cellulolytic activity [16]. The pathogenic fungi Rhizoctonia solani, Pythium ultimum, Phytophthora cactorum, Fusarium oxysporum, Dematophora necatrix were found in the soils of old dying gardens, which, along with bacteria and actinomycetes, are greater than in the soil of young gardens [14]. The aim of our research was to study the ratio of bacteria, yeast and fungi in soils occupied by apple orchards of different ages in the northern part of the Central Black Earth Region and fragmentarily in the Stavropol Territory.

2. Materials and methods
The research was carried out in 2015-2017 in fruit growing farms in the Tambov, Lipetsk, Penza regions and the Stavropol Territory. The objects of research were soils (typical chernozem, leached, podzolized, meadow-chernozem, chernozem-meadow, chernozem-moist-meadow and gray forest soils) under industrial apple orchards. The main tracts of chernozems typical for apple orchards were located in OJSC "Dubovoe" of Petrovsky region, OJSC fruit nursery "Zherdevsky" of Zherdevsky region and SEC "Zemlyansky" of Inzhavinsky region of Tambov region. The humus horizons (A + AB) of the soils had an average thickness, the groundwater level was low (9 m). The main area of chernozems leached under apple orchards was concentrated in the FSUE Michurinskoye, the Komsomolets breeding farm in the Michurinsky district, in the Dubovoye OJSC of the Petrovsky district, the Snezhetok AO of the Pervomaysky district and the Zherdevsky fruit nursery. In terms of the thickness of the humus horizons, the soils are thick, the groundwater level was low (7 m). The main part of podzolized chernozems under apple orchards was located in FSUE Michurinskoye and Planeta Sady Plus LLC in Michurinsky District and Snezhetok AOO in Tambov Region. The soils had an average thickness of humus horizons, the groundwater level was low (7 m). The main part of podzolized chernozems under apple orchards was located on the territory of FSUE Michurinskoye, JSC Uchkhozo-breeding plant Komskomolets, AOO Snezhetok and FNTs im. I.V. Michurin. The soils had thick humus horizons, the groundwater level was low (6.3 m). The main part of meadow-chernozem soils under apple orchards was located in the Territory of FSUE Michurinskoye, JSC Uchkhozo-breeding plant Komskomolets, AOO Snezhetok and FNTs im. I.V. Michurin. The soils had thick humus horizons, the groundwater level was low (6.3 m). The main part of meadow-chernozem soils under apple orchards was in LLC Planeta Sady Plus, SEC Zeleny Gai and Sady Michurina LLC (533 hectares), Snezhetok AO and Michurinskoe Federal State Unitary Enterprise of the Tambov Region. In terms of the thickness of the humus horizons, the soils are medium-thick, the groundwater level was low (4 m). The main massif of chernozem-meadow soils under apple orchards was located at FSUE Michurinskoye, Planeta Sady Plus LLC, Sady Michurina LLC, Zeleny Gai SEC of Michurinsky District and Snezhetok AO. The soils had thick humus horizons, the groundwater level was elevated (2.5 m). The largest area of chernozem-humid-meadow soils under apple orchards was available in Planeta Sady Plus LLC, Sady Michurina LLC, Zeleny Gai Agricultural Production Enterprise and Snezhetok AO. The soils had thin humus horizons, the groundwater level was high (50 cm). The main area of gray forest soils under apple orchards was located in the Michurinskoe Federal State Unitary Enterprise of the Michurinsky District of the Tambov Region and the CJSC
Agrofirm named after 15 years of October in the Lebedyansky District of the Lipetsk Region. The soils had humus horizons of reduced thickness, the groundwater level was low (6.3 m). Layouts of trees: 5x3, 6x4 and 6x8 m. Rootstocks: 62-396, 54-118 and seed.

Soil sampling was carried out according to the methodological instructions of V.V. Zerling and L.A. Egorova [1]. The number of microorganisms in the soil was determined by sowing on nutrient media at a dilution of 10,000 and 100,000 times [2].

3. Results and Discussion

We found that in waterlogged soils (chernozem-meadow and chernozem-moist-meadow), the greatest number of bacteria was noted, but the least number of fungi. The greatest amount of yeast and mold fungi, which are very valuable for the root system of the apple tree, is noted in forest soils (gray forest and podzolized chernozems) (table 1).

Table 1. The number of microorganisms in the 0-60 cm layer of different soil types in apple orchards of the Tambov and Lipetsk regions, thousand CFU/g (2015-2017).

| Soil type                  | Bacteria | Yeast         | Mold fungi |
|----------------------------|----------|---------------|------------|
| Typical black soil         | 8-10     | 2800-33000    | 2-10       |
| Leached chernozem          | 4-6      | 8000-50000    | 115-380    |
| Podzolized chernozem       | 4-8      | 2000-70000    | 1240-2500  |
| Meadow-chernozem           | 4-6      | 7000-44000    | 90-250     |
| Meadow-chernozem           | 4-6      | 6000-45000    | 80-200     |
| Chernozem-meadow           | 5-20     | 3600-40000    | 60-150     |
| Chernozem-wet-meadow       | 7-30     | 2500-38000    | 0.1-0.5    |
| Gray forest                | 4-8      | 1300-70000    | 550-900    |

It was found that in 1 g of leached chernozem of OJSC "Dubovoe" of the Tambov region in 2019, there were: 5-20 thousand CFU of bacteria, 3660-45785 thousand CFU of yeast and 60-890 thousand CFU of mushrooms in the 0-20 cm layer, in the layer 20-40 cm, respectively 4-8 thousand CFU of bacteria, 4350-9983 thousand CFU of yeast and 81-203 thousand CFU of fungi. Thus, the ratio "bacteria: yeast: fungi" in this soil in the 0-20 cm layer was 1: 1978: 38, and in the 20-40 cm layer - 1: 1194: 24. In the layer of 20-40 cm, in comparison with the upper layer, there were 2 times less bacteria, 3.4 times less yeast and 3.3 times less fungi.

In 1 years of the humus horizon of typical chernozem (with the presence of carbonates in the form of pseudo mycelium) from the apple orchard of the Rasskazovsky district of the Tambov region, in 2019, 10 thousand CFU of bacteria, 2490-33140 thousand CFU of yeast and 10 thousand CFU of fungi were contained.

In 1 years of chestnut soil of the Stavropol Territory from the garden, which killed apple trees contained 100 thousand CFU of bacteria, 4460 thousand CFU of yeast and 10 thousand CFU of fungi. In the same soil under normal trees, there were 25 thousand CFU of bacteria, 11240 thousand CFU of yeast and 20 thousand CFU of fungi. In 1 soil of horizon A from a very old apple orchard of the Penza region in 2018, there were 8 thousand CFU of bacteria, 1235 thousand CFU of yeast and 137.2 thousand CFU of fungi. In the same horizon of another section, there were 8 thousand bacteria, 70565 thousand CFU of yeast and 2500 thousand CFU of fungi.

A year after stubbing the orchard at Timiryazevo LLC in 2019, 1 g of leached chernozem was: 4 thousand CFU of bacteria in the 0-10 cm layer, 5970 thousand CFU of yeast and 115 thousand CFU of mushrooms, in the layer 10-40 cm respectively 6 thousand CFU of bacteria, 34704.5 thousand CFU of yeast and 373 thousand CFU of fungi.

The number of bacteria in the soil between rows increased with depth, and yeast and molds decreased. The number of bacteria and yeast in the soil of the near-trunk strips decreased with depth (table 2).
| Zone | Layer, cm | Number of bacteria | Yeast | Mold fungi |
|------|-----------|--------------------|-------|------------|
| Uchkhoz-breeding plant "Komsomolets". 28 year old garden. Tambov region | 0-10 | 18 | 3635 | 300 |
| Row spacing | 10-40 | 20 | 4330 | 10 |
| Barrel strip | 0-10 | 10 | 7660 | 650 |
| | 10-40 | 8 | 5400 | 69.7 |
| FNTS them. I.V. Michurin. 15 year old garden. Tambov region | 0-10 | 10 | 2535 | 310 |
| Row spacing | 10-40 | 11 | 1290 | 280 |
| Barrel strip | 0-10 | 6 | 27805 | 1917.6 |
| | 10-40 | 4 | 8395 | 453.8 |
| JSC "15 years of October". 28 year old garden. Lipetsk region | 0-10 | 25 | 6325 | 242.5 |
| Row spacing | 10-40 | 30 | 3727.5 | 345 |
| Barrel strip | 0-10 | 9 | 8032.5 | 547.5 |
| | 10-40 | 7.5 | 5290 | 455 |
| LLC Timiryazevo. 43 year old garden. Lipetsk region | 0-10 | 16 | 6900 | 140 |
| Row spacing | 10-40 | 22 | 3035 | 20 |
| Barrel strip | 0-10 | 8 | 48580 | 800 |
| | 10-40 | 6 | 30465 | 2031 |
| LLC Timiryazevo. 89 year old garden. Lipetsk region | 0-10 | 14 | 4055 | 85 |
| Row spacing | 10-40 | 21 | 5210 | 30 |
| | 40-50 | 15 | 1200 | 10 |
| | 0-10 | 10 | 9385 | 165 |
| Barrel strip | 10-40 | 10 | 8605 | 660 |
| | 40-50 | 8 | 1700 | 120 |

The soils of row spacing of apple orchards contained 2.3 times more bacteria, 3.8 times less yeast and 4.4 times less molds.

It was found that in the root layers of the soil of the near-stem strips of apple orchards that have entered full fruiting contains 6.8 CFU/g of bacteria, 12907 CFU/g of yeast and 572.8 CFU/g of fungi, that is, the ratio of "bacteria: yeast: fungi" will be 1: 1898: 84. In old apple orchards in the root zone of the soil of the near-stem strips, there were 8.3 CFU/g of bacteria, 28217 CFU/g of yeast and 1046 CFU/g of fungi, which is in a ratio of 1: 3400: 126.

4. Conclusion
Based on the foregoing:

- In the waterlogged chernozem-meadow and chernozem-moist-meadow soils, the highest number of bacteria was noted, but the lowest number of fungi. The greatest amount of yeast and molds (favorable for the apple rhizosphere micro flora) is noted in gray forest soils and podzolized chernozems.
- In the root layers of the near-stem strips of apple orchards, there were 2.3 times less bacteria, but 3.8 times more yeast and 4.4 times more fungi than in the aisles.
- There are more bacteria, yeast, and fungi in the humus horizon of the near-stem strips of old apple orchards than in the soils of younger orchards. As the age of the garden increases in the
ratio "bacteria: yeast: fungi", the proportion of yeast increases 2 times, and the proportion of fungi - 1.5 times.

- A year after stubbing the gardens in the 0-40 cm soil layer, the amount of molds becomes average between their content in the near-trunk strips and aisles.
- The death of trees on chestnut soil was noted where there were 5 times more bacteria, 2.5 times less yeast and 2 times less fungi.

References
[1] Zerling V V and Egorova L A 1980 Guidelines for the diagnosis of mineral nutrition of apple and other garden crops (Moscow: Kolos) 47
[2] Erismana F F 2004 Methods of microbiological control of soil. Methodological recommendations (Moscow: Federal Center for State Sanitary and Epidemiological Supervision of the Ministry of Health of the Russian Federation) 12
[3] Akintola A I, Oyedeji O, Bakare M K and Adewale I O 2017 Purification and characterization of thermostable cellulase from Enterobacter cloacae IP8 isolated from decayed plant leaf litter. Biocatalysis and biotransformation 35(5) 379-87
[4] Arafat Y, Din I U, Tayyab M, Jiang Y H, Jiang T, Chen Z Y, Cai H Y, Zhao X M, Lin W X, Lin S and Lin 2020 Soil Sickness in Aged Tea Plantation Is Associated With a Shift in Microbial Communities as a Result of Plant Polyphenol Accumulation in the Tea Gardens. Frontiers in plant science 11(601) 256
[5] Emenike C U, Liew W, Fahmi M G, Jalil K N, Pariathamby A and Hamid F S 2017 Optimal Removal of Heavy Metals From Leachate Contaminated Soil Using Bioaugmentation Process. Clean-soil air water 45(2) 1500802
[6] Erdem B, Dayangac A, Sahin I K 2017 The Presence of Metals and Antibiotics Resistant Bacteria in Arable Manure Soils. Acta physica polonica A 132(3) 570-1
[7] Ghaderitabar H, Mousavi A, Salmanian A H and Hadi F 2020 Novel aroA of Glyphosate-Tolerant Bacterium Pseudomonas sp. Strain HA-09 Isolated from Roundup-Contaminated Garden Soils in Iran. Iranian Journal of biotechnology 18(3) 80-7
[8] Gulidova V A, Kravchenko V A, Zakharov V L 2020 Optimization of agrophysical soil properties for spring rapeseed on leached chernozem. Amazonia Investiga 9(29) 63-8
[9] Hong S B, Geronimo F K, Choi S H and Kim L H 2018 Impacts of nonpoint source pollutants on microbial community in rain gardens. Chemosphere 209 20-7
[10] Inoue H, Okada A, Uenosono S, Suzuki M, Matsuyama T and Masaoka Y 2020 Does HLB Disease Prefer Citrus Growing in Alkaline Soil? Jarp-Japan agricultural research quarterly 54(1) 21-9
[11] Koberl M, Wagner P, Muller H, Ruetzler F, Cernava T and Berg G 2020 Unraveling the Complexity of Soil Microbiomes in a Large-Scale Study Subjected to Different Agricultural Management in Styria. Frontiers in microbiology 11 1052
[12] Ribbons R R, Kepfer-Rojas S, Kosawang C, Hansen O K, Ambus P, McDonald M, Grayston S J, Prescott C E and Vesterdal L 2018 Context-dependent tree species effects on soil nitrogen transformations and related microbial functional genes. Biogeochemistry 140(2) 145-60
[13] Shaharoon B, Al-Islainy S, Al-Mayahi A, Al-Harrasi N, Al-Kindi R, Al-Sulaimi A, Al-Busaidi H and Al-Abri M 2020 The role of urbanization in soil and groundwater contamination by heavy metals and pathogenic bacteria: A case study from Oman. Heliyon 5(5) 1771
[14] Singh N Sharma D P, Kaushal R, Sharma N, Sharma I M and Sharma S S 2020 Isolation and identification of fungi and nematodes in the rhizosphere soil of old declining apple orchards in Himachal Pradesh, India. Allelopathy journal 50(2) 139-52
[15] Wang Y J, Liu L, Luo Y, Awasthi M K, Yang J F, Duan Y M, Li H K and Zhao Z Y 2020 Mulching practices alter the bacterial-fungal community and network in favor of soil quality in a semiarid orchard system. Science of the total environment 725 138527
[16] Zakharov V L, Zubkova T V 2018 Assessing soil suitability for gardening in the north of the central black earth region using degradation data. *EurAsian Journal of BioSciences* **12** 1-8

[17] Zhang D, Ge S F, Wang C, Jiang Y M, Li X L, Xia S J, He J Z, Yao J, Zhang J N, Wane X Y 2020 The relationship between soil bacteria and metal nutrient availability for uptake of apple trees in Chinese orchards. *Plant growth regulation* **92(2)** 181-93

[18] Zhu Z.L, Y. Bai, M.L Lv, Tian G, Zhang X, Li L, Jiang Y M and Ge S F 2020 Soil Fertility, Microbial Biomass, and Microbial Functional Diversity Responses to Four Years Fertilization in an Apple Orchard in North China. *Horticultural plant journal* **6(4)** 223-30