Monitoring of Soil-Borne Pathogens in the Agricultural Soils of the Pestrechinsky District (Tatarstan, Russia)

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Abstract. A recent agricultural trend is aimed to develop organic farming technologies. Organic farming means no mineral fertilizers, pesticides, antibiotics and other chemical substances not characteristic of natural conditions should be used in farm production. When choosing the regions, where this technology can be successfully realized, it is important to evaluate not only the physical and chemical qualities of soils, but also the degree of their infestation with phytopathogens. The Pestrechinsky District of the Republic of Tatarstan, where transfer to organic farming is being planned, was chosen as such a region. Agricultural lands were marked at the map of the administrative region, 100 sampling site were generated using GIS Technologies. It was found out that soil microbial community was characterized by a typical ratio and count of yeasts fungi (3.4·10^6 – 1.6·10^7 CFU·g^{-1}), mold fungi (1.0·10^5 – 1.7·10^9 CFU·g^{-1}) and bacteria (1.6·10^8 – 3.1·10^9 CFU·g^{-1}). In all the selected soil samples plant pathogenic fungi of the Fusarium genus were found (26 to 250 CFU·g^{-1}), and as for another genus of plant pathogenic fungi, Alternaria, their count was rather low (0 to 9 CFU·g^{-1}), herewith in 46 samples out of 100 they were absent.

1. Introduction
A recent agricultural trend is aimed to develop organic farming technologies. Organic farming means no pesticides, toxic chemicals and mineral fertilizers having negative effect both on the soil covering and the soil microbial community should be used in farm production. All the measures listed above are used in so called conventional farming that, on the one hand, increases crop yields, but on the other hand caused soil degradation, soil and water pollution [1, 2, 3]. However, before organic farming can be implemented into practice, it is necessary to estimate the level of soil infestation. The Republic of Tatarstan is one of the leading subjects of the Russian Federation in the area of agriculture. Total cultivated area of the Republic is 3.7 mln ha, 52% out of which are under cereals (summer and winter wheat, rye, barley, oats, and pea), 37% – feed crop (maize, roots, and grass), 7% – industrial crops (sugar beet), 3% – vegetables and potatoes. But regions with the high level of industrial production are not suitable for the introduction of organic farming. Out of 43 municipal areas of the Republic about 15 municipal areas correspond to the necessary requirements. As the Pestrechinsky District is located close to the million city of Kazan and still have relatively favorable ecological situation, the government of the District decided to organize organic farming in it. Before the implementation of this technology into practice it is necessary to estimate the level of agricultural land infestation with phytopathogenic organisms in the region. This study is aimed to monitor the level of soil contamination with Fusarium genus and Alternaria genus in the Pestrechinsky District of the Republic of Tatarstan. Many Fusarium species are pathogenic microorganisms that may cause serious diseases in agricultural crops, garden or forest plants. Thus, some species of Fusarium oxysporum are
responsible for root rot or wilt in over a hundred species of plants [4, 5]. Some species, such as *F. graminearum*, *F. culmorum* or *F. avenaceum* lead to the destruction of grain crops, for example they cause *Fusarium* head blight in wheat. Being scientifically and economically important *F. graminearum* and *F. oxysporum* were included into the “Top 10” list of the most dangerous pathogenic fungi [6]. The species of *Fusarium* are also capable of producing mycotoxins, which contaminate agricultural crops, mostly cereals, and the food produced out of them. In wheat *F. graminearum* usually produce such trichothecenes as deoxynivalenol, and in maize *Fusarium verticillioides* produce fumonisin. These substances are classified as mycotoxins and they are harmful for the health of humans and animals [7]. The damage caused by *Alternaria* genus can be preharvest or postharvest. Cereals, vegetables and fruits may suffer [8]. Some *Alternaria* species not only spoil many articles of food directly, but also generate metabolites, which belong to the group of phytotoxins and mycotoxins, thus, *Alternaria* is harmful for plants, for animals and for people.

2. Material and methods

The present study was conducted on soils collected in the Pestrechinsky District located in the Republic of Tatarstan, Russia (N 55°45′, E 49°39′). A total number of 100 soil samples was selected in the agricultural lands to evaluate soil phytopathogenic properties. Sampling was carried out according to GOST 17.4.4.02-84 [9]. Sampling sites were chosen with the help of GIS Technologies. Landsat-8 satellite images dated 07/15/2013 were taken. The images were synthesized in pseudocolors (synthesis of 6, 5, and 4 channels); the procedure of synergism of the colored synthesized image with the resolution of 30 m and panchromatic channel with the resolution of 15 m was carried out. Thus, colored images were received with the spatial resolution of 15 m, which corresponds to the scale 1:50 000. The borders of agricultural lands of the Pestrechinsky Municipal District were digitized in EasyTrace software according to certain deciphering signs, such as the angularity of the borders of fields, the diversity of colors in the image, and distinctive colors. Using the vector layer received QGIS software generated 100 sampling sites in a random, and a rectangular buffer 100 m on a side was built for each sample (Figure 1).

![Figure 1. Map of the Pestrechinsky District, the Republic of Tatarstan, Showing Sampling Sites to Estimate Phytopathogenic Soil Characteristics](image-url)
Total microbial count was carried out using the method of serial dilutions in semisolid agar with further inoculation of a sample drop on the surface of a membrane filter. Determination of the count of yeast fungi and mold fungi was carried out using the method of inoculation of serial dilutions into Sabouraud’s medium, *Fusarium spp.* and *Alternaria spp.* plant pathogenic fungi count was determined by means of inoculation into agarized Czapek’s medium and further microscopic examination [10].

3. Results and Discussion
Microfungi and bacteria form the main component of soil microbial biomass, while as oozoae and archan comprise no more than 3% of it [11]. According to the ratio of fungi and bacteria in soil its condition can be determined [12]. The selected soil samples were characterized by typical cultivated bacteria content [10, 13, 14]; the data are presented in Figure 2. The lowest total microbial count was found in sample No 47 and was $1.6 \cdot 10^6$ CFU·g$^{-1}$, and the highest – in sample No 13 and was $3.1 \cdot 10^7$ CFU·g$^{-1}$. On average, $5 \cdot 10^6$ to $1 \cdot 10^7$ CFU·g$^{-1}$ bacteria were present in the examined agricultural soils of the Pestrechinsky District, 68 out of the 100 soil samples were characterized with such content of cultured bacteria.

**Figure 2.** Distribution of Samples According to the Total Microbial Count

Then the content of yeast fungi in soil samples was analyzed (Figure 3). The count of fungi in the samples varied from $3.4 \cdot 10^5$ CFU·g$^{-1}$ to $1.6 \cdot 10^6$ CFU·g$^{-1}$, herewith, in a big number of samples the count of yeast fungi was $6 \cdot 10^5$ CFU·g$^{-1}$ to $1 \cdot 10^6$ CFU·g$^{-1}$. Evaluating soils, Semenov et al. [15] found out that the typical ratio of fungi and bacteria in soil is 4-12, which corresponds to our results.

The count of mold fungi varied from $1.0 \cdot 10^4$ to $1.7 \cdot 10^5$ CFU·g$^{-1}$ (Figure 4). The most typical concentration of mold fungi in agricultural soils of the Pestrechinsky District was $0.6 \cdot 10^5$ CFU·g$^{-1}$ to $1 \cdot 10^5$ CFU·g$^{-1}$ (57 samples out of 100).

**Figure 3.** Distribution of Samples According to the Count of Yeast Fungi

**Figure 4.** Distribution of Samples According to the Count of Mold Fungi
*Fusarium* is considered to be one of the most destructive soil-borne diseases [16]. Wide spread occurrence of Fusarium diseases is connected both with the use of contaminated seed material, and with planting nursery transplants taken from contaminated greenhouse soils. The count of plant pathogenic fungi of the *Fusarium* genus in the samples analyzed varied from 26 to 250 CFU·g⁻¹ (Figure 5), whereby for 61 samples out of 100 the count of mold fungi was 60 CFU·g⁻¹ to 100 CFU·g⁻¹. It is worthwhile mentioning that no soil samples free from contamination with plant pathogenic fungi of the *Fusarium* genus were found. However, contamination of agricultural soils in the Pestrechinsky District cannot be considered high, as in the investigations carried out by Yao et al. [16] the total count of *Fusarium oxysporum* was high in the initial soil, reaching 17336 ± 2461 CFU·g⁻¹ dry soil.

![Figure 5. Distribution of Samples According to the Count of Plant Pathogenic Fungi of the *Fusarium* genus](image1.png)

![Figure 6. Distribution of Samples According to the Count of Plant Pathogenic Fungi of the *Alternaria* genus](image2.png)

*Alternaria* is a universal genus of plant pathogenic fungi including endophytic, saprobic and pathogenic species, which are able to survive on a wide range of hosts [17]. Some spaces of the genus are able to cause significant losses in many agricultural crops. Some taxa of *Alternaria* genus are characterized as postharvest pathogens dangerous for immuno-compromised patients, as they may cause phaeohyphomycosis in them. Besides, they produce airborne allergens. As *Alternaria* genus have serious negative effects on human health, it is very important for researches and other specialists to identify *Alternaria* species promptly. According to the results of our investigations, presence of *Alternaria* genus plant pathogenic fungi in the agricultural lands of the Pestrechinsky District is mostly not typical (Figure 6). The count of *Alternaria* genus plant pathogenic fungi was rather low (0 to 9 CFU·g⁻¹), herewith in 46 samples out of 100 they were absent.

**Conclusions**

In whole, the amount and the ratio of bacteria, yeast fungi and mold fungi in the tillable lands of the Pestrechinsky District are typical for agricultural soils. However, all the samples under analysis are contaminated with fungi of the *Fusarium* species. The count of plant pathogenic fungi of *Alternaria* species is low, or there are no fungi of these species. Thus, it is reasonable to realize organic farming on the tillable lands of the Pestrechinsky District in combination with biological methods of plant protection against *Fusarium* blight.

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