Soil suppressive activity as an indicator of its suitability for improved seed potato farming

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Abstract. The paper contains a description of the method, which allows assessing soil suitability for growing improved seed potatoes by the totality of microbiological characteristics of the soil. An indicator of the level of suppression of the soil microbial community is proposed as the main indicator of soil suitability, which is based on the species diversity of the soil microorganisms and its total suppressive activity against potato pathogens, in particular *Fusarium solani*. Among the six soil sites tested by this method at one of potato farms in Tomsk Region, virgin land sites, a site that had been in a fallow state for a long time, and an arable site that had been fertilized for several years were recognized as the most suitable for growing improved seed potatoes.

1. Introduction
Soil microorganisms are foundational for soil quality and health; they provide ecosystem services that are essential for agriculture, e.g. potato farming. It is a well-known that potatoes are highly affected by different diseases and often perish. This result in yield decline. Plant tissues, especially tubers, have a high level of nutrients, proteins and minerals. Thus, potatoes, like no other crop are a great breeding source for a phytopathogenic fungi growth such as *Fusarium solani*. They are a globally distributed fungal species causing vascular wilt, stunting and tuber rot. According to various scientific research, the total worldwide potato yield loss from different diseases is estimated about 30 - 40% of the annual harvest, in some cases this number is even higher, about 129 mln tons of the total yield production or 5 billion dollars. In Russia, the annual potato crop loss caused by diseases and plant pathologies is about 200 billion rubles as noticed by All-Russian Institute of Plant Protection [1, 2].

Nowadays, there is a shift towards using apical meristem to obtain healthy and virus-free mini-tubers as a seed material for growing potatoes in agro-developed countries, as a result of a high predisposition of potatoes to diseases. However, virus-free tubers are affected by pathogenic microorganisms on the second and third year of growing in soils with a high population of pathogens. This has resulted in a decrease in mini tubers usage, in particular, and in the effectiveness of the method as a whole.

So, in order to integrate the method of healthy mini-tubers usage as a widespread practice of potato growing, it is necessary to create a soil selection algorithm that allows finding the best microbiologically fitted areas. Thus, the aim of the research was to develop a unified soil microbiology assessment methodology that could be used for different potato farming regions.
The comparative in-vitro microbiome analysis was chosen as the main assessment method. The soil samples were collected from different areas with various agricultural regimes (e.g. 10 years of potato growing, fallow lands etc.). The main microbial indicators of soil quality used in this research were:

- the level of the microbial species activity in soils;
- the level of suppressive microbial activity in relation to *Fusarium solani*.

A number of studies have shown the correlation between soil microbial diversity and its quality. For example, Sokolov et al. (2009) proposed that Shennon index based on the soil microbiome could be used as a soil quality indicator [3]. Jan Frouz [4] and Aspen T. Reese [5] believed that microorganisms are the main factor of the pedogenesis which played a great role in plant growth and soil quality. Korobova (2010) noticed that microbiome vulnerability to minor ecological changes allows researchers to use microbiological indicators as a precise method of soil quality assessment.

2. Objects and methods

Soils were collected from six potato farming sites in Tomsk region. Different crop rotation was spotted in four studied areas with potato as the main crop and various levels of phytopathological structure (e.g. low and high prevalence and disease development). There were also two areas of virgin land that were adjoined to forest and arable lands. Soil was classified as luvisols (WRB classification) with clay loam texture. Soil samples were collected from the 0 - 20 cm depth in August. The short description of the studied sites could be found in Table 1.

### Table 1. Characteristics of the studied areas.

| Area, № | Sampling place | Cultivated culture | Predecessor | Potato yield | Level of potato disease affection | Potato persistence | Miscellaneous |
|---------|----------------|--------------------|-------------|--------------|-----------------------------------|--------------------|---------------|
| 1       | Arable land    | Wheat              | Potato      | Low          | High                              | Low                | —             |
| 2       | Arable land    | Wheat              | Potato      | High         | Low                               | High               | used organic fertilizers for 10 years* |
| 3       | virgin land    | Forb               | Forb        | —            | —                                 | —                  | Close to the area 2 |
| 4       | Arable land    | Potato             | Convertible husbandry | —    | —                                 | —                  | Set-aside area till 2015 |
| 5       | virgin land    | Forb               | Forb        | —            | —                                 | —                  | Close to the area 4 |
| 6       | Arable land    | Wheat              | Potato      | Low          | High                              | Low                | —             |

2.1. Determination of the microbial species diversity in soils

Soil samples for microbial analysis were collected from 7 - 10 spots that could make up representative data. Samples were collected in sterile containers using sterile instruments. Koch's pour plate method with beef-extract agar (BEA) was used to determine the total microbial number. The soil samples were dissolved in different ratio for the experiment \((10^{-3}, 10^{-4} \text{ and } 10^{-5})\). The BEA was diluted five times in order to expand the number of growing colonies. This method considerably increases the probability of growing colonies not only from *R*-strategy, but also from *K*-strategy. The plates were incubated at \(+20\ldots+22^\circ\text{C}\). Microbial colonies of different morphological types and their numbers were noticed after 10 days. The indices of soil microbial diversity were calculated. Therefore, to determine the level of species diversity the Simpson and Berger-Parker indices were used. The computation was based on the methodology of A.E. Magurran (1988) [8].
Since the determination of the microbial systematics and taxonomy is a highly time-consuming and enormously expensive process, we used Simpson and Berger-Parker indices which are widely known and distributed. Thus, the calculation was based on the frequency of the colonies of different microbial species rather than on the species itself. However, despite the fact that the spectrum of the detected species is narrowing, the cost and time efficiency of such techniques make this method highly beneficial.

2.2. Determination of the suppressive soil activity

The soil suppressive activity was based on the level of fungistatic activity of individual microbial strains. The strains were firstly isolated from the soils and later their fungistatic activity was analyzed in a test culture of the widespread potato pathogen – *Fusarium solani* fungi.

Four bacterial cultures were inoculated in the complex agar medium (BEA and potato-glucose agar 1:1) in Petri dishes. Strokes were symbolized by a square (Figure 1). Then the plates were incubated at +28°C for 24 hours. Blocks from the growing edge of 6-7-day old fungal colony were transferred to the center of the Petri dishes and were incubated for 6-7 days at +20...+22°C.

![Figure 1. The layout of the experiment (bacterial strokes and agar blocks with a fungi): 1, 2, 3, 4 – lines of the 4 bacterial culture; 5 – agar block with 6 days old fungi (*Fusarium solani*).](image)

The radius of the fungus was measured on each of the four lines drawn on the plate daily until the end of the experiment (up to 7 days). The growth rate was calculated as a straight-line tangent.

The average index of the inhibition rate was used to assess the soil suppressive activity. It was calculated as a percentage difference between the radial growth rate of the fungi towards the bacterial strokes and the growth rate in a plate without bacteria (control).

3. Results and discussion

3.1. Microbial properties and species diversity in the studied soils

The results of the *in vitro* experiments are shown in Table 2. The agro soils were characterized by the maximum number of the microorganisms (sites 1 and 6). The lowest numbers were observed in Petri dishes with soil samples from sites 2, 3 and 4. It was noteworthy that the difference between agro soils and virgin lands was not observed. For example, the total number of microorganisms in virgin lands is the same as that noticed in agricultural soils. The only noticeable similarity was found in the lower species diversity at virgin lands. The soils of sites 2, 3 and 5 are characterized by a low level of diversity of morphologically types of microbial colonies as it demonstrated in Table 1. The most microbiologically diverse soil sample was from site 4. This area had been in a fallow state for a long period of time and agricultural activity was started only in 2016.
To determine the species diversity, Simpson’s index is usually used. This coefficient describes the variability of microbial community, occurrence of the microorganisms and the taxonomy abundance. Simpson’s index calculated for the studied sites showed that the highest microbial diversity was typical for site 4 (Table 2).

The highest level of taxonomy diversity was noticed in soils from sites 4 and 5. It could be explained by a great plant diversity that resulted in more complex and taxonomic diversity of soil microbiome. Toropova et al. noticed that crop rotations can enhance disease suppressive capacity. It was demonstrated that the number of root rots negatively correlated with the saprotrophic microorganism activity and diversity [9, 10].

Berger-Parker diversity index reflects the richness and divergence of the microbial community. It equals the maximum value in the datasets and allows finding the dominant values. It often indicates the impact of external factors on the system, e.g. in ecologically oppressed areas with overgrazing and a low microbiological diversity in soils; or with prevalence of only dominant species.

The computation of Berger-Parker index for the soil microbial community allowed finding sites with the aligned values. Thus, these areas (sites 4 and 5 (Table 2)) are balanced and diverse in

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**Table 2.** The number of microorganisms formed morphologically different colonies on the beef-extract agar (CFU × 10^6 / g of totally dried soil sample), soil microbial biodiversity indices, the abundance of fungistatic active strains.

| Types of the microbial colonies* | Site 1 (arable land) | Site 2 (arable land) | Site 3 (virgin land) | Site 4 (arable land) | Site 5 (virgin land) | Site 6 (arable land) |
|---------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                                 | Number               | species abundance    | Number               | species abundance    | Number               | species abundance    |
| 1                               | 0.3                  | –                    | 0.7                  | –                    | 0.3                  | –                    |
| 2                               | 2.9                  | –                    | 0.4                  | –                    | 14.8                 | 37.5                 |
| 3                               | 28.2                 | –                    | 2.4                  | 1.6                  | 0.7                  | 1.6                  |
| 4                               | 7.9                  | –                    | 1.04                 | –                    | 0.3                  | 21.5                 |
| 5                               | 38.0                 | –                    | 4.8                  | 2.7                  | 1.3                  | 3.3                  |
| 6                               | 131.0                | –                    | 35.9                 | 19.8                 | 20.5                 | 51.9                 |
| 7                               | 13.1                 | –                    | 6.6                  | –                    | –                    | 1.4                  |
| 8                               | 0.3                  | –                    | 0.7                  | 0.2                  | –                    | 3.1                  |
| 9                               | 3.6                  | –                    | 1.4                  | –                    | –                    | 0.3                  |
| 10                              | –                    | 0.3                  | –                    | –                    | 0.3                  | 0.6                  |
| 11                              | –                    | 0.4                  | –                    | –                    | 0.3                  | 0.6                  |
| 12                              | –                    | –                    | 0.4                  | –                    | –                    | 21.5                 |
| 13                              | –                    | –                    | 0.4                  | –                    | 0.3                  | 0.6                  |
| 14                              | –                    | –                    | 0.4                  | –                    | –                    | 21.5                 |
| 15                              | –                    | –                    | 0.4                  | –                    | 0.3                  | 0.6                  |
| 16                              | –                    | –                    | 0.4                  | –                    | 2.0                  | 1.4                  |
| 17                              | –                    | –                    | 0.4                  | –                    | 10.7                 | 19.1                 |
| Total number of the microorganisms | 181.5                | 39.6                 | 29.5                 | 56.2                 | 146.9                | 177.4                |
| Simpson diversity index         | 0.53                 | 0.73                 | 0.53                 | 0.38                 | 0.42                 | 0.68                 |
| Berger-Parker index             | 1.27                 | 1.40                 | 2.00                 | 2.70                 | 2.75                 | 4.43                 |

Note.* – the same numbering of colony morphotypes for different sites does not mean the identity of the morphotypes themselves and the identity of the taxonomic affiliation of the microorganisms on which they formed.
microbiome composition. Consequently, it could be assumed that the environmental and phytosanitary conditions are suitable for potato farming in these areas.

3.2. Suppressive soil activity towards test-culture of the potato pathogen microorganism at the studied sites

The suppressive soil activity is one of the instructive integral indicators of soil health assessment. Thus, it can be used as a useful factor of the soil ecological and phytosanitary quality evaluation [11, 12]. Lobmann et al. considered the soil suppressive activity as an individual disease suppression parameter and withdrew it from the general soil microbiome properties [13].

We assessed the suppressive soil activity as an average value of fungistatic activity of microbial strains isolated from the studied soils towards *Fusarium solani*. The suppression of bacterial strains is illustrated in Figure 2. The radial growth towards two bacterial strains is shorter than in the control Petri dish.

![Figure 2](image)

**Figure 2.** The inhibition of the radial growth of *Fusarium solani* by metabolic activities of bacterial strains, isolated from soils from site 4.

The radial growth rates of *Fusarium solani* were determined using daily observations and comparison with control. The values of *Fusarium solani* growth were calculated as a straight-line tangent towards the bacterial strokes. The calculation example is demonstrated in Figure 3.

![Figure 3](image)

**Figure 3.** The radial growth rate of *Fusarium solani* in biotests with bacterial strains isolated from arable soil samples from site 4.

Note. The control is a line describing the fungi growth on a Petri dish without bacteria. 4.4 … 4.12 are lines that describe the fungi growth rate on a similar medium towards the corresponding bacterial strokes.

Furthermore, the inhibition (suppressive) coefficient (*K*) was calculated. It was determined for all studied sites and for each fungal colony, in particular. The following formula was used to calculate the suppressive coefficient.

\[
K = \frac{V_i \times 100 \%}{V_k}
\]
Where: $K$ – inhibition rate, %; $V_i$ – growth rate towards the bacterial strokes, mm/hour; $V_k$ – growth rate of the fungi in control, mm/hour.

The level of fungistatic activity of individual bacterial strains varied significantly: from zero suppression rate to 60% (Table 3).

**Table 3.** The inhibitor coefficients of the *Fusarium solani* radial growth on the studied soil samples.

| Strain, № | Site 1 (arable land) | Site 2 (arable land) | Site 3 (virgin land) | Site 4 (arable land) | Site 5 (virgin land) | Site 6 (arable land) |
|-----------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| 1         | 11.6                 | ---*                 | ---*                 | ---*                 | 17.1                 | 22.5                 |
| 2         | 8.5                  | ---*                 | 27.9                 | ---*                 | 17.8                 | 14.7                 |
| 3         | ---*                | 23.6                 | 24.0                 | ---*                 | 17.9                 | 10.9                 |
| 4         | 11.63                | 19.7                 | ---*                 | 18.6                 | 24.03                | 21.7                 |
| 5         | 8.5                  | 31.0                 | 24.1                 | 10.9                 | 24.0                 | 17.0                 |
| 6         | 5.4                  | 27.9                 | 22.5                 | 16.3                 | 29.3                 | 8.5                  |
| 7         | 13.9                 | ---*                 | ---*                 | 17.0                 | 2.3                  |                      |
| 8         | 14.0                 | 24.0                 | 5.4                  | 31.0                 | 6.9                  |                      |
| 9         | 13.2                 | 13.5                 | 1.10                 | 7.9                  | 35.7                 |                      |
| 10        | 11.8                 |                      |                      | 27.0                 | 59.4                 |                      |
| 11        | 13.5                 |                      |                      | 23.3                 | 20.8                 |                      |
| 12        | 8.3                  |                      |                      | 20.2                 | 53.3                 |                      |
| 13        | 16.2                 |                      |                      | 1.2                  | 8.7                  |                      |
| 14        |                      |                      |                      | 45.4                 | 15.3                 |                      |
| 15        |                      |                      |                      | 48.5                 | 7.4                  |                      |
| 16        |                      |                      |                      | 12.7                 | 3.6                  |                      |
| 17        |                      |                      |                      | 1.1                  |                      |                      |
| Average   | 11.38                | 23.28                | 17.5                 | 20.07                | 21.69                | 19.29                |

Note. * – there is no suitable data due to the strain development properties which are not allowed to make a straight line suitable for the experiment (the colony penetrates the substrate or spreads all over the substrate, forms rhizoid spikes, etc.). The strains with the highest fungistatic activity are in bold.

The average inhibition rates were calculated for each strain and soil sample (Figure 4).

**Figure 4.** Suppressive soil activity in relation to *Fusarium solani*: * – difference between the suppressive soil activity noticed for sites 2 - 6 is correlated with the data from site 1 and verified by Student’s t-test ($p<0.05$).
As it is shown in Figure 4, the lowest inhibition rate (11%) of *Fusarium solani* was spotted in the soils from site 1. The highest level of the suppressive activity was noticed in the soil samples from sites 2 and 5.

3.3. **Integral indicator of the level of suppression of potato pathogens by the microbial community of the soil of the studied sites**

The inhibition rate coefficient based on the average inhibition level for individual microbial strains could have some errors because the abundance of the strains was not used in its calculations. Moreover, the index of taxonomic diversity should be an integral part of the suppressive activity assessment.

Thus, as a more precise and error-free indicator of specific site suitability for potato farming, we developed the **soil suppression indicator**. The methodology of the suppression indicator evaluation is shown below. It was used for soils from potato farming fields in Tomsk region.

Let us suppose that the lowest level of the fungistatic activity of bacterial strains, when it could be part of the suppressive soil activity, is 20%. These values are shown in bold in Table 5. The soils from site 1 are not suitable for the subsequent analysis as the inhibition rate is less than 20%, and the area is not acceptable for the modified potato farming.

The strain abundance was evaluated for the soils collected from 5 studied areas. The previous microbiological *in vitro* analysis provides data for computation (Table 2).

The strain abundance was calculated using the following formula.

\[ SA = \frac{N_i \times 100\%}{N} \]  

Where:
- \( SA \) – species (strain) abundance, %; 
- \( N_i \) – strain number, CFU/1 g of the dried soil; 
- \( N \) – total number of strains in soils, CFU/g of the dried soil.

Then the indicator of soil suppression was calculated based on the \( SA \).

\[ S = \ln \left( \sum (SA_i \times K_i) \times \frac{1}{D} \right) \]  

Where:
- \( S \) – soil suppression indicator; 
- \( SA_i \) – abundance value for each active strain, %; 
- \( K_i \) – value of the inhibition rate of fungal colony by each active strain, %; 
- \( 1/D \) – Simpson’s species diversity index.

For example, for the soils from site 2, the suppression indicator is 6.66.

\[ S = \ln \left( (37.8 + 83.7 + 552.4 + 4.8) \times 1.15 \right) = \ln (780.5) = 6.66 \]

Then we calculated the level of suppression for all studied sites in the same way. The results are shown in Table 4.

**Table 4. Soil suppression index (*Fusarium solani*) for different studied sites.**

| Site, № | 1 (arable land) | 2 (arable land) | 3 (virgin soil) | 4 (arable land after convertible husbandry) | 5 (virgin soil) | 6 (arable land) |
|---------|-----------------|----------------|----------------|------------------------------------------|----------------|----------------|
| Soil suppression index (S) | 0 | 6.66 | 8.05 | 8.67 | 8.68 | 5.74 |

Furthermore, the composition of the original soil bacterial community and effects of management practices coinciding with the hypothesis proposed previously. Consequently, as it demonstrated in Table 6, soils from sites 3, 5 and 4 (area after the fallow stage) were characterized by the highest level of suppression activity. Some middle values were spotted for site 2. We believe that it was caused by the regular use of organic fertilizers as also confirmed by Sokolov [14], Zinchenko and Stoyanova [15]. Thus, it could be possible to use site 2 for potato farming after remediation measures. Sites 6 and 1 have the lowest soil suppression indicator and could not be used for potato farming since the disease development probability is high for this environment.
4. Conclusions

- The developed methodology of the complex suppressive activity evaluation allows predicting the suitability of soils for improved potato cultivation.
- Among the studied areas, the most suitable sites for potato farming were sites 3, 5 (virgin land) and 4. The last one was under the fallow for many years, which resulted in the highest level of microbiome diversity in the soil samples collected from this area. The suppressive soil activity is the highest for all 3 sites.
- The arable land (site 2), where organic fertilizers were applied for several years, in the future, after a set of agrotechnical measures aimed at reducing the effect of soil fatigue, can also be used to grow seed potatoes.

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