Evaluation of Suppressiveness of chernozem upon the intake of organic fertilizers under the conditions of a model experiment

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Abstract. The problem of maintaining the sanitary function of soils of agroecosystems is one of the main ones, since it determines the danger for reproduction of various types of microorganisms, including human and animal pathogens. Within the study, the authors assessed the number of bacteria (Salmonella enterica and Escherichia coli) in samples of chernozem with introduction of organic fertilizers. The dynamics of the number of Salmonella enterica shows that organic matter in soils contributes to a sharp decrease in their number at the initial stages of biological pollution, and in the long term it contributes to total loss. By the 60th day, Salmonella was found only in the control option of the experiment, but continuation of the experiment showed their total loss on the 80th day. Introduction of organic ameliorants into the chernozem samples led to a decrease in the number of E. coli by the 80th day, but their total loss was not noted.

1 Introduction

It is known that microorganisms in agricultural soils have a significant effect on the level of soil fertility, especially in terms of the availability of plant nutrients and suppression of pathogenic microflora carried by the soil [1].

These key ecological soil functions will depend on critical constituents of the soil microbial community. In fact, the "health" of the soil can be defined in terms of its ability to counteract (suppress) pathogenic microflora – suppressiveness [2].

The topic of studying soil suppressiveness is actively discussed by scientists from all over the world. Among Russian scientists, the papers of E.Yu. Toropova, M.S. Sokolov, A.P. Glinushkin, who studied induction of soil suppressiveness, are highlighted, and among foreign scientists, the papers of G.A. Toranzos, R.P. Marcos, who studied bacteria pathogenic for humans, transmitted through the soil are highlighted.

Suppressiveness has been conceptually divided into "general" and "specific" one, where general suppressiveness is the result of the vital activity of many organisms. (for example,

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as a result of competition for habitat or nutrients), and specific suppressiveness is manifested in the antagonism of certain types of bacteria or fungi [3].

Approaches to increasing the suppressiveness of agricultural soils shall include the analysis and management of a wide range of factors [4].

Environmental factors that determine the "health" of a particular soil type include climatic variables such as rainfall, moisture and temperature, as well as physicochemical and biological activity indicators. Agronomic parameters can include types and varieties of crops, sowing dates, sequence of crop rotation and soil methods [5]. Other parameters may include the use of agrochemicals such as pesticides, growth regulators, fertilizers and ameliorants [6].

Microorganisms are another variable to consider when making agricultural decisions. The potential damage caused to plants by phytopathogenic microorganisms has a significant impact on the choice of crops and varieties of agricultural crops, crop rotations, planting density and timing, seed treatment and the list of agricultural chemicals used [7].

Studies aimed at identifying microorganisms involved in specific suppression face a number of methodological problems: first, the number of species inhabiting the soil is often very large, and estimates range from thousands to millions; secondly, most microorganisms are difficult to cultivate using standard methods and to assess their antagonistic interactions with pathogenic flora [8].

Alternatively, the grower may choose to knowingly use beneficial microorganisms when treating seeds or fertilizing the soil. For example, rhizobia and mycorrhiza can increase plant productivity, while many epiphytic microorganisms can enhance plant defenses [9].

While general suppression refers to suppression of pathogens resulting from the maximum amount of microbial activity, specific suppression refers to the antagonistic activity against a pathogen by individual microorganisms or a more narrowly defined group of organisms. One of the key points in implementing the potential of soils to reduce the number of pathogenic microorganisms is to determine their species, requirements for soil conditions and resistance [10].

Determining the number of both the pathogen and the organisms that suppress it will allow the development of more effective methods for predicting the potential danger of soils as a source of infection. The totality of the knowledge gained will make it possible to develop new strategies for combating pathogenic soil microflora by using various agricultural techniques, incl. application of organic and bacterial fertilizers. [11]. This determines the relevance of our paper.

The aim of the study was to assess the effect of organic fertilizers on the suppressiveness of chernozems in relation to *Salmonella enterica* and *Escherichia coli* under the conditions of a model experiment.

2 Materials and Methods

The objects of the study were 2 types of enterobacteria *Salmonella enterica* and *Escherichia coli*). The choice of study objects is determined by their high occurrence as agents of biological pollution of environmental components and well preserved in highly humic and mineral types of soils [12, 13].

Study materials: southern chernozem soils selected in the territory of the Park named after Perovsky, Orenburg (coordinates No:51.7718500, Eo:55.0934390). The following was used as organic fertilizers: soil conditioner Reasil Soil Conditioner, Super Gazon (NPO SILA ZHIZNI, Saratov) and biohumus AgroVerm (Manufacturer BioEraGroup, Moscow).

In the course of the work, we used classical microbiological methods: sampling method and preparation, soil treatment for bacteriological analysis; methods for isolating and accounting for bacteria in soil (R. Koch's method); method of limiting dilutions and sowing on solid nutrient media.

For the experiment, the selected soil was pre-dried, sieved and sterilized at a temperature of 120 °C within 40 minutes. Therefore, weighed portions of 27 g were taken and introduced into sterile Petri dishes. After that, solutions of organic ameliorants – soil conditioner and vermicompost at a dose recommended by the manufacturer were added to the soil. The soil without the addition of organic ameliorant served as a control.

Then the soils were kept for a week in a thermostat at a temperature of 25 °C, and then 10 ml of a suspension of bacteria (*Salmonella enterica* or *Escherichia coli*) at a concentration of 10^9 CFU/ml according to the turbidity standard (McFarland) was added to each Petri dish (Figure 1).

THE SOIL	EXPERIENCE OPTIONS: 1. CONTROL (WITHOUT ADDING PREPARATIONS). 2. BIOHUMUS. 3. SOIL REMOVER-SHITEL	ADD 10 ML (109 CFU / ML) BACTERIA SUSPENSION (SALMONELLA ENTERICA / ESCHERICHIA COLI) IN SIZE SOLUTION	SEEDING ON FOOD AND FOOD MEDIA FOR 10, 20, 40, 60 AND 80 DAYS

Fig. 1. Scheme of the experiment

After contamination, the soil was placed in a thermostat at a temperature of 25 °C and for the period of 10, 20, 40, 60, and 80 days, the number of bacteria was assessed by inoculation on elective media. BTN agar was used to isolate E. coli, and bismuth sulfite agar was used for Salmonella. Sowing was carried out in 7-10 fold repetition using standard identifiers.

3 Results and Discussions

3.1 Influence of organic fertilizers on some soil properties

The organic fertilizers taken for the experiments were characterized by a high content of organic matter, and the AgroVerm preparation was characterized by a sharply alkaline reaction of the medium (pH 11).

Organic fertilizers are an important factor in changing the content of soil organic matter, and also affect the complex of its agronomic properties. Changes in the chemical properties of soils with introduction of organic ameliorants affects the survival of pathogenic and opportunistic microorganisms [14, 15].

The results of determining the pH showed that introduction into the soil of biohumus AgroVerm and the soil conditioner Reasil Soil Conditioner, Super Gazon, did not lead to a change in the reaction of the soil solution (Table 1).

Table 1. Change in pH and humus content when adding ameliorants

Sample	pH_{H2O}	$C_{org.}$, %
Control	7.15±0.03	5.6±0.03
Biohumus	7.18±0.07	7.5±0.1
Soil conditioner	7.16±0.02	8.2±0.07

However, at the same time, an increase in the content of organic matter was noted with addition of vermicompost to 7.5 %, and a soil conditioner — to 8.2 %.

3.2 Salmonella enterica Population Dynamics in Chernozems

Enterobacteriaceae are among the most common microorganisms that cause acute intestinal diseases. They can serve as an indicator of biological pollution of lands of settlements and agricultural purposes. Bacteria actively enter the external environment with human and animal feces. They multiply in the soil in presence of a large amount of organic matter, but they can multiply even in clean water [16].

The results of determining the abundance of *S. enterica* show that after 10 days of the experiment, the option of the soil sample with a soil conditioner was the best suppressor of bacteria (Figure 2).

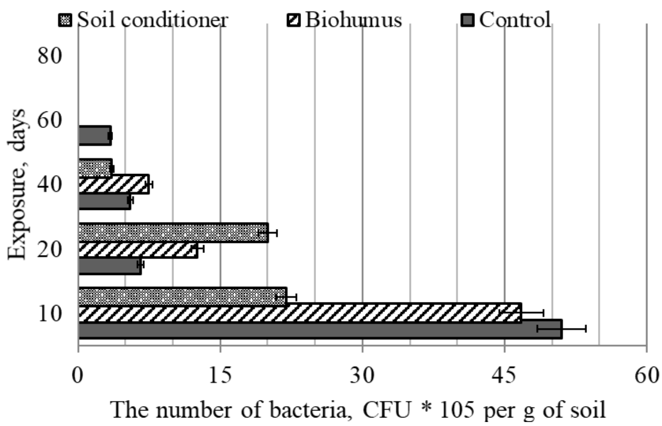

Fig. 2. Dynamics of the number of Salmonella enterica in samples of chernozem

The number of bacteria in the sample with addition of a soil improver was 22.2•10^5 CFU/g soil on the 10th day of the experiment and decreased to 20.1•10^5 on the 40th day, and on the 60th and 80th days of the experiment, bacteria in the soil were not detected.

In the option with introduction of vermicompost, the number by 10 days was 47•10^5 CFU/g, and with an increase in the exposure of the experiment, it decreased to 7•10^5 CFU/g by 40 days, further studies showed the absence of viable *Salmonella enterica* cells in the samples of chernozem.

In the control option of the experiment, there was a sharp decrease in the number of Salmonella from 51•10^5 CFU/g to 7•10^5 CFU/g from 10 days to 5•10^5 CFU/g by 20 days. This was the only option of the experiment, where bacteria remained on the 60th day, and by the 80th day, Salmonella was not detected, as in other options of the experiment.

3.3 Changes in the Abundance of Escherichia coli in Soil Samples

Presence of microorganisms of the genus *Escherichia* in soils, including the type species *Escherichia coli*, is an indicator of fresh faecal contamination. To identify *Escherichia coli*, biochemical tests and elective media are used, which ensure the predominant growth of some organisms over others. E. coli is one of the indicators of the sanitary state of soils [17].

In control sample of soil on day 10 the number of bacteria was 46•10^5 CFU/g (Figure 3). Then, an increase in the duration of the experiment contributed to an increase in the number of bacteria to 136•10^5 CFU/g, and a further increase in the exposure time contributed to a decrease in the number with a minimum value of 15•10^5 CFU/g by 80 days.

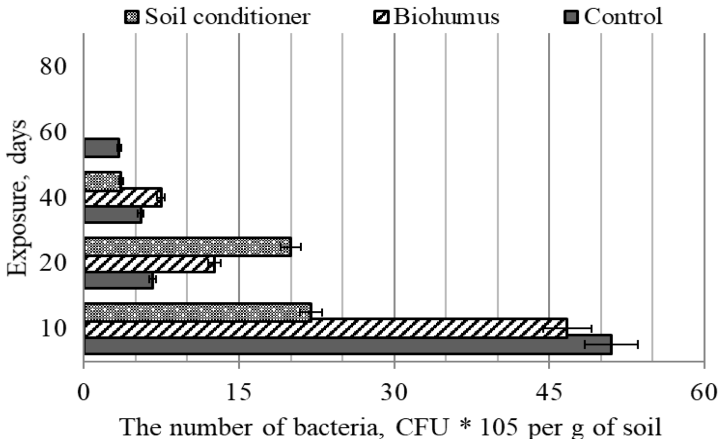

Fig. 3. Dynamics of survival of Escherichia coli in soils

The number of Escherichia coli in the option with introduction of vermicompost on the 10th day was 44•10^5 CFU/g soil, and by the 20th and 40th days it increased almost 2 times. Further dynamics of the number is associated with a decrease in their number with a minimum value of 14•10^5 CFU/g.

Analysis of the results of the experiment in the option of applying the soil improver showed a decrease in the number of bacteria by 73 % during the entire period of the experiment.

The main feature of soil contamination with *Escherichia coli* was preservation of viability by 80 days, i.e. total loss of bacteria is not observed.

3.4 Analysis of Results

According to studies by Sugiyama et al. (2010), Njira et al. (2013), and Silvina et al. (2019), a close relationship was found between the survival of pathogenic and opportunistic microflora representatives with the content of organic matter, soluble humus and salts in the soil. Therefore, we set the task of identifying the dependence of the number of bacteria on the content of organic matter and pH, which changed with introduction of organic ameliorants. The results of calculating the Spearman correlation coefficient are presented in Table 2.

The results of the correlation analysis showed that the indicator of the number of Salmonella is characterized by a high negative correlation with the exposure period of the experiment, which suggests that the older the pollution, the less likely the bacteria will retain their viability in the chernozem.

They also turned out to be more sensitive to the indicator of organic carbon content, it follows from this that the more organic matter in the soil, the less likely it is for bacteria to remain in the soil.

Table 2. Correlation analysis

Indicators	The number of *S. enterica*, CFU/g	The number of *E. coli*, CFU/g
Exposure time, days	-0.76	-0.23
pH_{H2O}	-0.54	-0.2
$C_{org.}$, %	-0.73	-0.57

A similar relationship was noted for *E. coli* only between the indices of abundance and the content of organic matter, but it is weaker in comparison with the analogous values of the correlation coefficient for Salmonella.

4 Conclusion

The results of the study led to several conclusions:

1. Introduction of organic fertilizers into the soil practically did not affect the pH of the soil solution, but led to an increase in the content of organic matter with addition of vermicompost to 7.5 %, and the soil conditioner to 8.2 %.
2. The dynamics of the number of *Salmonella enterica* shows that organic matter in soils contributes to a sharp decrease in their number at the initial stages of biological pollution, and in the long term it contributes to total loss. By the 60th day, Salmonella was found only in the control option of the experiment, but continuation of the experiment showed their total loss on the 80th day.
3. Introduction of organic ameliorants into the chernozem samples led to a decrease in the number of *E. coli* by the 80th day, but their total loss was not observed.
4. The indicator of the number of *S. enterica* is characterized by presence of a high negative correlation (r^2 = - 0.76, at p = 0.038) with the indicator of the exposure period of the experiment and the content of organic carbon (r^2 = - 0.73, at p = 0.042), which indicates that the older the pollution, the less likely it is that bacteria will retain their viability in the chernozem, as well as the more rapid decrease in their number when organic ameliorants are introduced into the soil.
5. A weak correlation was found for the indicator of *E. coli* abundance with pH and organic carbon content, which confirms the thesis of its greater resistance to external environmental factors and the ability to survive in soils for a long time.

Author’s contributions

Lyudmila Galaktionova, Tatyana Vasilyeva and Svyatoslav Lebedev conceived and planned the experiments. Gulnur Yamansarina carried out the experiments.

Zhanara Sulimenova contributed to the interpretation of the results, contributed to sample preparation.

Lyudmila Galaktionova and Gulnur Yamansarina took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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