The effect of different doses of pig manure on soil microbiological activity and spring wheat yield

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Abstract. The effect of the application of different norms of liquid pig manure on microflora composition and microbiological activity of leached chernozem, as well as on its sanitary and microbiological indicators, has been studied. It was found that at the maximum application rate, the number of bacteria increased to a greater extent than that of actinomycetes and fungi. At the same time, the genera Pseudomonas, Cellvibrio, and Bacillus predominate among the bacteria. Micromycetes of the genus Mukor and Aspergillus predominate among fungi. The nitrate content remained within acceptable limits, the mineralization coefficient for this variant is quite high. According to sanitary and microbiological indicators, when pig manure is applied in the maximum norm, the samples fall into the group of heavily polluted soil. According to the index of the coli -titer for enterobacteria, all the studied soil samples fall into the group of slightly polluted, the sanitary and epidemiological situation has not worsened. The application of the maximum manure rate had a positive effect on wheat yield.

1. Introduction
The activity of populations of microorganisms associated with the manure of farm animals is an issue which relevance goes beyond the agricultural industry. Accordingly, the manure produced by animals on the enterprise territories is concentrated to such an extent that the nutrients in its composition can no longer return to the natural cycle of substances in a timely manner due to natural environmental mechanisms.

Problems associated with the accumulation of manure in the vicinity of agricultural enterprises can manifest themselves in different ways. Sometimes the negative effect on the environment from organic-rich manure runoff created by livestock complexes exceeds the environmental damage caused by harmful emissions from large industrial enterprises. Manure runoffs are particularly dangerous for reservoirs [1].

Pig-breeding enterprises create the most acute problem of air pollution with odor-forming substances. Such substances consistently cause irritation, discomfort, and deterioration of people well-being. There is an opinion that it is the problem of unpleasant odor in the future that will become the main deterrent in pig breeding development [2].

A no less problem is the deterioration of the sanitary situation when excessive amounts of manure accumulate on the territory. If infectious diseases of animals occur on the farm, then the manure will be contaminated with their pathogens. But even if such cases do not occur, the content of pathogenic bacteria, viruses, fungal spores will still increase in the environment with manure accumulation [3, 4].
The highest diversity of anaerobic microorganisms is in fresh manure, in manure runoffs it is somewhat lower [5]. It is noted that fresh manure is a medium for the preservation of Mycobacterium tuberculosis, and the virulence of this disease pathogen under favorable environmental conditions can persist for more than a year [6]. If manure is brought into the territory regularly, the situation only worsens. This is manifested in the fact that the elimination rate of dangerous disease pathogens in the soil decreases, a longer period is required for its self-purification [7].

Each ton of fresh manure contains millions of weed seeds, which negatively affects the phytosanitary condition of fields when it is applied without special preparation [4].

Thus, the storage of manure requires compliance with special rules [8]. Liquid manure runoffs require complex disinfection. In practice, existing systems do not always meet the requirements of environmental protection [9].

On the other hand, if a rational approach is applied to animal manure disposal, then this production waste can be usefully applied in different directions [1]. First, when it is applied to the fields, the removal of nitrogen with the crop is compensated, as well as other chemical elements. This compensation mechanism is an integral part of the organic farming concept [10]. With a scientifically based approach to the application of these organic fertilizers, they provide a stable increase in yield. Methods of manure technical utilization (filtration, drying) have been developed. The severity of the problem of an excessive number of dangerous microorganisms can be reduced by artificial acidification of the substrate to a pH of 5.3 [10]. The volatilization of nitrogen in the form of ammonia can be reduced by adding grain straw to the manure mass [10]. Manure can be part of a vermicompost (warm manure) used to neutralize sewage sludge using California worms. In this case, manure will contribute to reducing the severity of the industrial pollution problem [11, 12]. In addition, manure is an integral component of the substrate for growing champignons, and the urea in its composition can be used to control the parameters of oyster mushroom fruit bodies [13].

For the destruction of manure runoff, bacterial-based biopreparations have been increasingly introduced into production in recent years, turning it into an environmentally safe material [4].

2. Methods
The research was carried out in the steppe zone of the Chelyabinsk region in the Uvelsky district. In autumn, liquid pig manure was introduced in the fields near pig breeding complexes. Fertilizers were not applied for control in some fields, in other areas manure was applied at a rate of 200, 250 and 300 t/ha. In the spring, spring wheat was sown on untreated and control (without fertilizers) fields. Soil samples were taken in the arable horizon during the tillering phase of spring wheat. The samples were analyzed on the day of sampling.

Based on microbiological studies, the sanitary situation of the soil, the composition of qualitative and quantitative microflora were evaluated. The studied material was seeded on standard nutrient media with subsequent identification by serial dilution method. The counting of the grown colonies was carried out by the agar plate method. When considering the results, the average number of colonies that grew during the sowing of each breeding was determined. Fixed preparations were prepared for bacterioscopic studies, stained by Gram and examined with an immersion system. Micromycetes were isolated by standard mycological methods. The diagnosis of pathogenic enterobacteria was carried out on the nutrient medium agar endo-GRM.

3. Results and discussion
3.1 The effect of pig manure on soil microbiological activity
The question of the effect of organic fertilizers on the soil microbiological activity is well covered in the literature. The processes of plant biomass transformation, humus formation and mineralization of organic matter occur with the direct participation of soil microorganisms and lead to the creation of a water-resistant structure, the granulometric composition of the soil and the optimal ratio of plant
nutrition elements [14]. The significant increase in soil biogenicity and the intensity of microbiological processes when using pig manure can be found in the works of many authors [15].

According to Table 1, when applying the studied norms of fertilizers in the soil, the presence of all groups of microorganisms increased, but to varying degrees, for example, the number of bacteria at the maximum norm (300 t/ha) increased 2.4 times compared to the control, where fertilizers were not used, in actinomycetes - 1.9 times, fungi - 1.6 times, respectively. From this it can be concluded that bacteria are most actively involved in nitrogen mineralization compared to other groups of microorganisms.

### Table 1. The number and ratio of individual groups of microorganisms after pig manure application.

| No. | Fertilizer rate | Total number of microorganisms in 1 g of soil | Number of microorganisms / percentage of total, % | SAA/MPA |
|-----|----------------|---------------------------------------------|-----------------------------------------------|---------|
|     |                |                                             | bacteria | actinomycetes | fungi |         |
| 1   | Control (without fertilizer) | 18.18 x 10⁵ | 13.8 x 10⁴ | 3.3 x 10⁴ | 10.8 x 10⁴ | 1.47 |
| 2   | 200 t/ha       | 23.42 x 10⁵ | 18.1 x 10⁴ | 4.0 x 10⁴ | 13.2 x 10⁴ | 1.41 |
| 3   | 250 t/ha       | 34.78 x 10⁵ | 28.6 x 10⁴ | 4.6 x 10⁵ | 15.6 x 10⁴ | 1.27 |
| 4   | 300 t/ha       | 41.31 x 10⁵ | 33.4 x 10⁵ | 6.2 x 10⁵ | 17.1 x 10⁴ | 1.90 |
|     | LSDₜ₅         | 5.00 x 10⁵  |                                             |         |         |       |

Cultivation on meat-peptone agar makes it possible to identify soil bacteria - ammonifiers capable of converting organic forms of nitrogen. Starch-ammonia agar is used to isolate microorganisms that utilize mineral nitrogen. At the same time, the ratio of the number of amylolytic microorganisms to ammonifying ones (SAA/MPA) serves as a mineralization coefficient indicating the intensity of processes in the soil [16].

When applying liquid pig manure, as well as with an increase in its application rate, the content of microorganisms in the soil increases, both ammonifying (MPA medium) and amylolytic (SAA medium). At the same time, the number of ammonifiers at the maximum manure rate increased by 2.3 times relative to the control, and amylolytic - ones by 3 times. Also, when the maximum norm was introduced, the content of all forms of nitrogen increased several times, ammonium by 2.5 times, nitrate by 5 times, respectively.

### 3.2 The effect of pig manure on the qualitative and quantitative composition of soil microorganisms

Intensive development of pig breeding complexes is impossible without solving the issues of efficient and environmentally safe disposal of organic waste, the volume of which is so large that the only way of processing is to use them as organic fertilizer. This leads to a change not only in the soil nutrition regime, but also in the overall state of the soil-biotic complex, which ensures its quality and safety.

The intensity of pig manure conversion into organic fertilizer by microorganisms depends not only on the ratio of fractions, the composition of feed, litter and the type of animals, but also on the ratio and quantity of ammonifiers, nitrifiers, denitrifiers, mold fungi, the sources of which are not only the internal microflora of animals [5], but also the soil microflora. The climate and abundant vegetation create such conditions in chernozem soils that the number of microorganisms can reach 3.5 million microbial cells in 1 g of soil.

Tables 2 and 3 show the number and ratio of individual groups of microorganisms in the soil when using pig manure.

During the conducted studies, it was found that the number of non-spore-forming rod-shaped
ammonifiers of the genus *Pseudomonas* increases with the introduction of pig manure, with a maximum norm of 2.7 times compared to the control. Bacteria of the genus *Micrococcus* involved in the decomposition of organic residues containing proteins with the formation of ammonium most often began to occur in variants with the introduction of organic fertilizers.

Gram-positive anaerobic cocci of the genus *Sarcina* were found in a variant with the application of manure 300 t/ha. In the studied soil, regardless of the manure application, cellulose-decomposing vibrios of the genus *Cellvibrio* and rod-shaped bacterium of the genus *Cytophaga* were found in different proportions, most likely characteristic of this soil, but the introduction of manure activated their activity, therefore the number increased.

Since microbiological analysis of soil samples was carried out in a dried state, the vast majority of grown bacteria were represented by the genus *Bacillus*. Spore-forming prokaryotes denitrifiers of the genus *Bacillus subtilis*, *B. megaterium*, *B. mesentericus*, *B. mycoides* involved in the stage of the process of reducing nitrates to gaseous oxides of molecular nitrogen were most often noted in variants with the introduction of organic fertilizers. The number of CFU of microaerophilic, gram-positive *Bacillus brevis* involved in redox reactions, mobilizing the intensity of soil respiration most of all, was met with an increase in the norm of pig manure. *Bacillus simplex* - gram-positive mobile aerobes most resistant to adverse effects were found in all variants of the experiment in a ratio independent of the fertilizer application rate. Gram-positive curved bacilli *Bacillus idosus* in small quantities were found in variants with the highest rate of manure application.

**Table 2.** Species composition and number of microorganisms, CFU, n-10⁵/g.

| No. | Fertilizer rate | Genus of microorganisms |
|-----|-----------------|-------------------------|
|     |                 | *Pseudomonas* | *Cytophaga* | *Cellvibrio* | *Micrococcus* | *Sarcina* | *B. megaterium* | *B. subtilis* | *B. brevis* | *B. simplex* | *B. mycoides* | *B. mesentericus* | *B. idosus* | *Actinomyces* | *Mycobacterium* |
| 1   | Control (without fertilizers) | 2.5 | 1.5 | 2.4 | 1.1 | n/a | 1.0 | 1.4 | 0.9 | 2.1 | n/a | 0.9 | n/a | 3.3 | n/a |
| 2   | 200 t/ha       | 2.7 | 2.3 | 2.9 | 2.3 | n/a | 2.0 | 2.4 | 2.7 | 0.8 | n/a | n/a | 2.9 | 1.1 |
| 3   | 250 t/ha       | 4.2 | 2.4 | 4.7 | 4.2 | n/a | 1.9 | 2.1 | 2.6 | 1.8 | 1.7 | 2.0 | 1.0 | 2.2 | 2.4 |
| 4   | 300 t/ha       | 5.8 | 2.0 | 4.0 | 4.1 | 1.0 | 2.6 | 2.7 | 3.6 | 2.2 | 2.0 | 2.1 | 1.3 | 1.8 | 4.4 |

The number of actinomycetes of the genus *Actinomyces* is the largest in the control variant, as the dose of organic fertilizer increases, their number decreases, they are probably competitors with prokaryotes, which are activated with the greatest dehydration. Actinomycetes of the genus *Mycobacterium*, on the contrary, began to become more active as pig manure was introduced, and their number increased as its norm increased.

It is well known that fungi dominate in relatively nitrogen-poor soils with low fertility. During the conducted studies, it was found that the share of fungal microflora accounts for 4-6% (Table 1), while their greatest number is in the version without fertilizers, where fungi do not have to compete with bacteria. A small proportion of fungi is still a positive thing, since there are many phytopathogenic forms in this group in relation to grain crops.

While establishing the taxonomic affiliation of fungi to a particular genus presented in Table 3, it turned out that the frequency of occurrence of non-pathogenic and non-toxic yeasts of the genus *Saccharomyces* and *Candida* in dried soil samples is insignificant and does not depend on the rate of application of organic fertilizers.

Saprophytic micromycetes of the genus *Mukor and Aspergillus penetrate the substrate in the upper
soil layer, settle on the remains of living organisms and turn them into humus, their number has increased with an increase in the norm of pig manure. Mold fungi of the genus *Penicillium* destroy various bacteria in the soil, but due to the increase in their numbers with the introduction of manure, the frequency of occurrence of the genus *Penicillium* decreases.

Fungi of the genus *Fusarium* persist in the soil, on plant residues, in the plants, they can infect plants, causing pathological changes, when applying manure at a rate of 300 t/ha they were not detected, most likely due to the greatest microbiological activity. In other variants, the number of fusariums increased as the soil was saturated with organic matter. *Trichoderma* suppresses the development of pathogens of seed, root, and soil infections, as well as diseases of fruits and leaves.

### Table 3. Species composition and abundance of CFU fungi, n·10⁴/g.

| No. | Fertilizer rate | Genus of fungi | micromycetes | yeast fungi |
|-----|-----------------|----------------|--------------|------------|
|     |                 |                | **Mucor**    | **Aspergillus** | **Penicillium** | **Fusarium** | **Trichoderma** | **Alternaria** | **Saccharomyces** | **Candida** |
| 1   | Control (without fertilizers) | 5.2 | 2.4 | 1.3 | 0.8 | 0.3 | 0.2 | n/a |
| 2   | 200 t/ha        | 4.3 | 3.1 | 1.1 | 1.2 | 1.1 | 2.4 | n/a | 0.3 |
| 3   | 250 t/ha        | 7.0 | 5.2 | 0.9 | 2.5 | n/a | n/a | 0.2 | n/a |
| 4   | 300 t/ha        | 9.6 | 6.5 | 1.0 | n/a | n/a | n/a | n/a | 0.1 |

The genera of the fungus *Alternaria* are mainly represented by saprophytic and phytopathogenic species involved in the decomposition and mineralization of dead plants. In variants where fertilizers were not applied and where alternaria and trichoderma were applied at a minimum dose, but with the activation of competitive ammonifiers, these micromycetes were not detected.

### 3.3 The effect of pig manure on sanitary and microbiological indicators of leached chernozem

The use of organic fertilizers increases the number and activates the biological activity of the soil microflora, which depends on the timing of application, dose, and methods of embedding in the soil. Biological features of pig manure depend on the number and ratio of bacteria, fungi, actinomycetes, unicellular algae, viruses, helminth eggs, weed seeds, most of which pose an ecological and agrotechnical danger.

The quality and degree of soil safety in epidemic and hygienic terms for humans and animals determines its sanitary condition. The use of untreated animal husbandry waste, especially liquid waste, is particularly dangerous from a sanitary point of view. The microbial number used to characterize soil contamination should not exceed 1-1.5 million individuals in 1 g of sanitary-clean soil.

Indirect indicators are sanitary-indicative microorganisms, namely coliform bacteria (BGC), *Cl. Perfringens*, bacteria from the genus *Proteus*, thermophilic and nitrifying bacteria. Their presence in the soil indicates its fecal contamination.

According to the data obtained, the microbial number in the studied soil samples varied from 1.7 to 4.0 million per 1 g of soil. It was revealed that at the control and when applying 200 t/ha, the microbial number corresponds to the category of slightly polluted soil, and at the norms of 250 and 300 t/ha, the samples fall into the group of heavily polluted soil.

According to the index of the coli-titer for enterobacteria grown on the endo-agar medium, all the
studied soil samples fall into the group slightly polluted soil. At the same time, regardless of the application rate of pig manure, the coli-titer for enterobacteria exceeds 1, which characterizes the studied soil according to the BGC titer as clean, when applying the maximum fertilizer rate, the titer of enterobacteria decreased by 3.5 times relative to the control.

3.4 The effect of pig manure on the spring wheat yield
High microbiological activity of the soil due to the use of organic fertilizers characterizes high soil fertility and ensures high crop yields. Moreover, the use of organic fertilizers causes an increase in yield within two to three years, unlike mineral fertilizers, which have practically no aftereffect [17].

According to the data, the yield of wheat grain consistently increased with an increase in the application rate of organic fertilizers. At the maximum rate, it was 2.27 t/ha, which is 0.76 more than in the absence of fertilizers at the control. The dependence of the yield on the fertilizer rate turned out to be higher on the mineralization coefficient, which in some cases decreased with an increase in the fertilizer rate, it follows that the mineralization coefficient can be considered as a particular indicator that does not reflect the full picture of the supply of plants with nutrients. At the same time, yield is a complex indicator that allows to get an accurate idea of plant nutrition level. This indicator reached the highest value at the maximum rate of fertilizers, it amounted to 2.27 t/ha.

4. Conclusions
When applying the studied norms of fertilizers in the soil, the presence of all groups of microorganisms increased, but to varying degrees, bacteria are most actively involved in nitrogen mineralization compared to other groups of microorganisms, at a maximum rate of 300 t/ha, the number of bacteria increased 2.4 times compared to the control, where fertilizers were not used, in actinomycetes - 1.9 times, fungi - 1.6 times, respectively. The application of pig manure contributed to an increase in the number of bacteria and actinomycetes, with a fertilizer rate of 300 t/ha, the genera Pseudomonas, Cellvibrio, Bacillus predominate among bacteria, their number was 5.8, 4.1 and 16.5 x 10^5 CFU in 1 g of soil, respectively. Fungi account for 4-6% of the total number of microorganisms, among them micromycetes of the genus Mukor and Aspergillus predominate 9.6 and 6.5 x 10^4 CFU/g.

According to sanitary and microbiological indicators, when applying pig manure 200 t/ha, the microbial number corresponds to the category of slightly polluted soil, at the norms of 250 and 300 t/ha, samples fall into the group of heavily polluted soil. According to the index of the coli-titer for enterobacteria, all the studied soil samples fall into the slightly polluted group.

Thus, the sanitary and epidemiological situation during the introduction of liquid pig manure has not worsened, but the number of microorganisms involved in the mineralization of organic matter has increased and, accordingly, the mineral nutrition of spring wheat has improved, which allowed, at a rate of 300 t/ha, to increase grain yield from 1.51 to 2.27 t/ha.

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