Influence of Pollution by Antibiotics on Biological Properties of Soils (Through the Example of Ordinary Chernozem)

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Original Research

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Abstract

In model laboratory and field conditions, the influence of pollution by antibiotics (benzylpenicillin, ampicillin, streptomycin, oxytetracycline, tylosin, pharmasin, tromexin, aliseryl, and nystatin) on the biological properties of ordinary chernozem was examined in concentrations of 1-1000 mg/kg. A decrease in the majority of the basic biological parameters of chernozem occurs when the concentration of antibiotics is 100 mg/kg of soil. In most cases, there was a direct relationship between the content of antibiotics in the soil and the scale of a decrease in the studied parameters. The degree of the influence of antibiotics was determined by their nature, concentration and time of exposure. Antibacterial antibiotics had more negative impact on the studied indicators than fungicidal ones. By the degree of inhibiting the biological properties of chernozem, antibiotics formed the following sequence: ampicillin > benzylpenicillin ≥ streptomycin ≥ oxytetracycline > tylosin ≥ pharmasin > nystatin > tromexin > aliseryl. Among the examined biological parameters when polluted by antibiotics, the most informative one was the number of ammonifying bacteria and the activity of dehydrogenases. The least informative was the indicator of catalase activity. The abundance of bacteria of the genus *Azotobacter* in case of pollution by antibiotics was not informative. The degree of a decrease in biological indicators was more pronounced in laboratory conditions than in the field ones. The rate of the biological activity recovery of chernozem after pollution in the field was 2 times higher. According to the degree of resistance to antibiotics, the investigated microorganisms of chernozem formed the following sequence: bacteria of the genus *Azotobacter* > micromycetes > amylolytic bacteria > ammonifying bacteria. Enzymes formed the following sequence: peroxidase ≥ polyphenol oxidase > catalase > dehydrogenase > invertase ≥ phosphatase. Antibiotics had prolonged influence on the biological properties of ordinary chernozem. The examined parameters were observed not to be recovered to control values even on the 120th day after the pollution.

Introduction

Unlike pesticides, which have long been used in agriculture, antibiotics have not been of interest as potential pollutants of the environment. But given the intensification of their use in all spheres of agriculture, special attention at present is given to the problem of polluting natural ecosystems by antibiotics.

As knowledge about antibiotics deepened, data on the adverse effects of uncontrolled use of these drugs began to be accumulated. The data of the research company *Research Techart* (2013) testified that approximately 3.5 thousand tons of antibiotics were used annually in Russia, 36% of them were used as antiparasitic drugs, 23% – for animals' treatment and disease prevention, 19% – as growth stimulants, and 22% – as prophylactic agents.

The Central Authority for Environmental Protection of Germany investigated the extent of using antibiotics in the European Union (EU). It turned out that animal industries were the main consumers of antibiotics. One of the first countries that initiated the ban on the use of antibiotics in the animal industry was Sweden, followed by Switzerland, the Netherlands and several other European countries. In Russia, feed antibiotics are not prohibited. The EU countries still do not have any environmental standards regarding tetracyclines and sulfonamides. Many attempts are made today to fill the gaps in scientific knowledge about the behavior and distribution of antibiotics in the environment and, in accordance with this knowledge, to make amendments in legislation (World Health Organization ..., 2014).

An important ecological consequence of the reckless use of antibiotics in agriculture is an increase in their background concentrations. In general, antibiotics get into the soil due to the application of manure (Golet *et al.*, 2003; Kemper, 2008) and sewage (Thiele-Bruhn, 2003; Renew, & Huang, 2004) on agricultural land as a fertilizer. Every year, more and more often different concentrations of antibiotics are found in sewage, soil, groundwater and drinking water (Schwartz *et al.*, 2003; Radjenovic´ *et al.*, 2009; Rosal *et al.*, 2010; Yang *et al.*, 2011; Zhou *et al.*, 2011). Locally in soils, there are antibiotics of the tetracycline group in concentrations of 1-900 mg/kg (Winckler, & Grafe, 2001; Pawelzick *et al.*, 2004), macrolide group - in concentrations of 1-800 mg/kg (Hamscher *et al.*, 2002; Höper *et al.*, 2002).

Currently, agricultural workers are accused of the fact that antibiotics from agricultural lands get into nearby water bodies and then into soils. Municipal water purification systems cannot filter out antibiotics. In connection with the above-said, it is important to study how pollution by antibiotics affects the state and functioning of ecosystems and soil cover, in particular.

The purpose of the study is to reveal the regularities of the influence of pollution by antibiotics on the biological properties of soils (on the example of ordinary chernozem) in laboratory and field conditions.

Materials And Methods

Chemicals

To study the consequences of pollution of ordinary chernozem by antibiotics, the following antibiotics were chosen: bactericidal antibiotics (benzylpenicillin, ampicillin, streptomycin), bacteriostatic antibiotics (oxytetracycline, tylosin, pharmasin), antibiotics used as growth stimulators in animal industry (tromexin, aliseryl), fungicidal antibiotics (nystatin). Full information on antibiotics is in the database on medicines http://drugreg.ru/Bases/.

Soil

The object of the study was ordinary chernozem of the South European facies (Botanical Garden, Southern Federal University), carbonate, powerful, slightly humic, heavy clay on yellow-brown and loessial loam (Table 1). The investigated type of soils was chosen in connection with the fact that chernozems constituted the majority of the soil cover of the south of Russia and were of particular importance in the country's food supply. The humus horizon thickness of the used chernozem was about 80 cm, the granulometric composition was heavy loamy, the reaction of environment was 7.7, the humus content was 4.1%, the total nitrogen (according to Kjeldahl) was 0.25%, total phosphorus (according to Ginzburg et al.) – 0.18, labile phosphorus (according to Machigin) – 28.8, total potassium (according to Berzelius) – 2.06% (Valkov et al., 2008).

Experimental test procedure

Laboratory model research

The soil for laboratory model research was selected from the arable layer (0-25 cm). Air-dry soil samples were treated with solutions of medical antibiotic – benzylpenicillin, veterinary antibiotic – pharmasin - and its mixture with fungicidal antibiotic nystatin in a wide range of concentrations of 1-1000 mg/kg of soil. Biological properties were studied on the 10th, 60th, and 120th days of incubation. The concentrations used were selected from literature data according to the residual amounts of antibiotics found in the environment (Christian *et al.,* 2003; Grote *et al*., 2004; Thiele-Bruhn *et al*., 2004; Kay *et al*., 2005; Sarmah *et al*., 2006; Sun *et al*., 2014). Then the effect of medical antibiotics was studied – ampicillin, streptomycin, and veterinary antibiotics – tylosin, tromexin, aliseryl in a concentration of 500 mg/kg of soil, using the results of previous reconnaissance studies (Akimenko *et al*., 2014, 2015). Biological indicators were examined on the 3rd, 30th and 90th day of incubation. All soil samples were incubated at a temperature of 20-25 °C and optimum moistening (60% of the field moisture capacity). The control was soil, not polluted by antibiotics.

Field model research

In the field model experiments, the plots were laid according to the generally accepted method of field experimentation (Field Methods..., 1967). We used a plot of 1 m^2. Repeatability was threefold. To study the dynamics of changes in biological properties, the soil samples were collected on the 3rd, 30th and 60th days after administering antibiotics. Antibiotics (oxytetracycline, tylosin) were introduced into the soil in the form of solutions in a concentration of 500 mg/kg of soil. Control areas were not treated with antibiotics. Within the framework of the field modeling, the influence of antibiotics on the growth and development of peas was studied, and the "Aksaiskii usatyi 7" variety was selected from *Cirrosut vulgatum*. Antibiotics were introduced into the soil by watering, after seed germination on the 10th day. The control was the plots sown with peas, without antibiotics.

Laboratory and analytical methods of research

Laboratory and analytical studies were conducted at the Department of Ecology and Nature Management of the Southern Federal University, using methods, which were common in ecology, biology, and soil science (Galstyan, 1978; Khaziev, 1991; Kazeev, & Kolesnikov, 2012). A comprehensive study of the microbiological properties of ordinary chernozem included determining the total number of bacteria by the method of luminescence microscopy with acridine orange staining, as well as the number of viable microorganisms of various ecological and trophic groups by the method of seeding the corresponding dilutions into solid nutrient medium. The enzymatic activity of the soil was judged by the activity of enzymes of the class of oxidases and hydrolases. The catalase and dehydrogenase activity was determined by the methods of A.Sh. Galstyan (1978), invertase activity – by the modified colorimetric method of F.Kh. Khaziev (1991), the activity of phosphatase – by the method of A.Sh. Galstyan and E.A. Arutyunyan (1966), the activity of peroxidase and polyphenol oxidase – by the method of L.A. Karyagina and N.A. Mikhailova (1986). The medium reaction (pH) was determined by the potentiometric method in a soil suspension of 1:2.5. The peas' indicators were studied by the following morphometric parameters and components of the crop structure: total phytomass (g), total number of pods (pcs.), average length of pods (mm), total weight of pods (g), weight of pods without peas (g), the average number of peas in pods (pcs.), the total weight of peas (g), the weight of 100 peas (g) and yield (dt/ha).

The biological properties of soil were analytically determined in 3-fold repetition for studying the microbiological properties of soils and in 4-9-fold repetition for studying the biochemical properties of soils.

To determine the general regularities of the influence of antibiotic pollution on the biological condition of chernozem, an integral indicator of the biological state (IIBS) of the soil was used. IIBS was determined by the most informative biological indicators (Kazeev, & Kolesnikov, 2012). From microbiological ones, the number of ammonifiers, amylolitic enzymes and micromycetes was taken into account, from biochemical ones – the activity of enzymes of the class of oxidases (catalase, dehydrogenase) and hydrolases (invertase, phosphatase) was used. To calculate the IIBS of the soil, the value of each of the selected indicators was taken as 100% (in the control and unpolluted soil sample) and in relation to the control, the values of other indicators (in polluted soil) were reflected in percentage terms. Then the average value of the selected indicators for each option was determined. This technique of calculation allows combining the relative values of various indicators, the absolute values of which cannot be summed up, since they have different units of measurement.

Statistical processing of research results

Since the biological properties of soils vary widely (Kazeev, & Kolesnikov, 2012), the biological values obtained in the work were subjected to statistical variation analysis, dispersion and correlation analysis. Statistical processing of data was conducted using statistical package Statistica 10.0. The following main indicators of the variation statistics were calculated: average ($M \pm m$), the coefficient of variation (*CV*), standard deviation (s). The sensitivity of the studied indicator was evaluated according to the degree of its decrease depending on the concentration of the antibiotic, and the information content – according to the degree of value correlation of the indicator and the concentration of the antibiotic.

Results

Based on the results of laboratory modeling of the pollution of chernozem, the negative effect of antibiotics and their combinations on biological parameters was established (Fig.1-2, Table 2). The degree of decrease in indicators depended on the nature of the antibiotic, the concentration, and the duration of exposure. In most cases, there was a direct relationship between the content of antibiotics in the soil and the degree of decrease in the studied parameters (correlation between the concentration of antibiotics and the decrease in the investigated parameters was r = –0.68=-0.86). From the range of selected concentrations of 1-1000 mg/kg, a minimal statistically significant effect of the inhibiting influence of antibiotics was observed at a concentration of 100 mg/kg of soil. On the subsequent timeframes of the study, the tendency of recovery of the number of microorganisms was observed. Ammonifying bacteria appeared to be more sensitive to introduced antibiotics than amylolytic bacteria and micromycetes. The abundance of bacteria of the genus *Azotobacter* was reduced only with the introduction of high concentrations of antibiotics (from 500 mg/kg).

Using the method of luminescent microscopy, the author established a decrease in the total number of bacteria with pollution of chernozem by antibiotics (Table 2). A close correlation was established between the concentration of antibiotics and the total number of bacteria (r = –0.85). The greatest decrease in the total number of bacteria occurred in the first 10 days from the moment of pollution. At a later date, there was a tendency to restore their numbers. Nevertheless, despite the observed tendency, and even on the 120th day after the pollution, the difference in the number of bacteria in the polluted and control samples was 23% (1000 mg/kg of soil ($p < 0.01$).

The maximum inhibition of enzymatic activity was observed in the first 10 days after the pollution by antibiotics; at later stages, there was a tendency to restore their activity (Table 3). A correlation analysis of the obtained data revealed a positive correlation of enzymes of two classes (dehydrogenase, invertase) with the number of micromycetes (r = 0.63, r = 0.65, respectively), catalase with ammonifying bacteria (r = 0.73), and inverse correlation of phosphatase with amylolytic bacteria (r = –0.80). This makes it possible to make judgments regarding the contribution of the group of microorganisms to the enzymatic pool of soils. Apart from enzymatic activity, when chernozem is polluted by antibiotics, the parameters of the reaction of the medium (pH). Antibacterial antibiotics lead to a slight shift of pH to the alkaline direction, fungicidal ones – to the acid direction. It seems most likely that the change in pH is related to the fact that antibiotics themselves are decomposed into chemical constituents and metabolites, which can contribute to changing the acidity of soils.

The field modeling results of chernozem pollution by antibiotics (tylosin, oxytetracycline) are presented in dynamics in Figures 3-4 and Table 4.

The analysis of the dynamics of biological parameters of ordinary chernozem showed that the maximum effect of pollution by antibiotics was manifested at the first stages from the moment of pollution. After that, there was a tendency to restore the indicators. The microbiological indicators of chernozem varied to a greater extent than biochemical indicators. Ammonifying bacteria, as well as in the framework of laboratory modeling, proved to be less resistant to pollution by antibiotics than other studied groups of microorganisms (Fig. 3).

The enzymatic activity of soils also changed (Fig. 4). The activity of hydrolases changed to a greater extent, compared to oxidases. Among the class of oxidases, antibiotics reduced the activity of dehydrogenase to a greater extent. The maximum decrease in catalase activity was established on the 3rd day of the study (by 18% from the control). Further there was a tendency to restore the activity of an enzyme. Among the studied enzymes, antibiotics did not affect the activity of peroxidase and polyphenol oxidase, but on the contrary, there was a tendency that the activity of these enzymes at all stages of the study increased. A negative correlation was established between these enzymes with the number of ammonifying bacteria (r = –0.82), and a positive correlation - with micromycetes (r = 0.75). The decrease in hydrolases' activity was observed on the 3rd day of the study, and in the subsequent periods, there was a slight increase in enzymatic activity in relation to the control.

The results of field modeling showed that antibiotics practically did not affect the state of vegetative organs of peas and their morphological indicators (Table 4). Despite this, the indicators of the state of generative organs, including yield, differed from the control values by a factor of 1.5. Among the studied antibiotics, oxytetracycline had greater effect on the growth and development of peas than tylosin.

According to the results of field modeling of chernozem pollution by antibiotics, a decrease in the integral indicator of the biological state of the chernozem was established. The greatest differences in relation to the control were established on the 3rd day of the experiment. The introduction of both tylosin and oxytetracycline led to decrease in the IIBS by 16 and 17%, respectively. In the following stages, there was a tendency of the increase in the IIBS. On the 30th day, the difference compared to the control was 8% for tylosin and 7% for oxytetracycline. On the 60th day of the IIBS study, chernozem in variants with tylosin and oxytetracycline approached the control (by 4% for tylosin, by 6% for oxytetracycline). The IIBS of soil while peas cultivation varied insignificantly.

The maximum inhibition of biological indicators by antibiotics, both in laboratory and in the field conditions, occurs in the first periods after pollution, and thereafter a tendency is observed for their recovery. Most antibiotics inhibit the number of ammonifying bacteria (Fig. 5). Amylolytic bacteria and micromycetes are relatively resistant to antibiotics.

The degree of decrease in biochemical indicators, namely enzymatic activity, was more pronounced in laboratory conditions than in the field. The activity of the examined enzymes recovered more rapidly in the field than in the laboratory. In addition, a high degree of variability of indicators in the field conditions was noted. Among the enzymes of the oxidoreductase class, dehydrogenase activity changed the most with antibiotics' pollution, among hydrolase – invertase activity changed the most. Statistically significant changes in the activity of peroxidase and polyphenol oxidase were not established.

For the IIBS of chernozem, both in laboratory and field conditions, the same tendencies were established. At the initial stages, after pollution by antibiotics, there was a decrease in the IIBS of chernozem; further, there was a tendency for the increase in IIBS (Fig. 6). In laboratory conditions, the degree of reduction of IIBS was more pronounced than in the field ones.

Discussion

The negative effect of antibiotics and their combinations on the biological properties of ordinary chernozem has been established. In conditions of acute pollution, the maximum effect of antibiotics was observed in the first 10 days from the moment of pollution. The following increase in the concentration of antibiotic causes the development of microorganisms' resistance (Rysz, & Alvarez, 2004; Schmitt *et al.*, 2004). From the ecological point of view, the question whether, in the course of remediating polluted habitats, resistant microorganisms could be replaced with antibiotic-sensitive populations, restoring original characteristics, was of interest (Alonso et al., 2001). Antibacterial antibiotics had greater negative impact on the studied indicators than the fungicidal ones. By the degree of inhibiting the biological properties of chernozem, antibiotics formed the following sequence: ampicillin > benzylpenicillin ≥ streptomycin ≥ oxytetracycline > tylosin ≥ pharmasin > nystatin > tromexin > aliseryl. Among the examined biological parameters when polluted by antibiotics, the most informative one was the number of soil microorganisms (ammonifying bacteria) and the activity of dehydrogenase. The least informative was the indicator of catalase activity. The abundance of bacteria of the genus *Azotobacter* in case of pollution by antibiotics was not informative. The degree of decrease in biological indicators was more pronounced in laboratory conditions than in the field ones.

Pollution of chernozem by antibiotics leads to a change in the enzymatic activity indicators. As the concentration increases, the inhibiting effect of antibiotics is enhanced. Similar to the results obtained, other studies have shown that antibiotics of the tetracycline series at a concentration of 300 mg/kg inhibit the activity of soil enzymes (catalase and phosphatase) by 35-55% (Feng *et al.*, 2009). Mixtures of antibiotics have a more pronounced effect of inhibiting soil enzymes. For example, a mixture of pharmasin + nystatin led to decrease in the activity of catalase, dehydrogenase and phosphatase by over 50% from the control ($p < 0.001$) on the 10th day of the experiment. This indicated possible synergistic effect of antibiotics interaction. Other studies (Cernohorska, & Votava, 2008; Feng et al., 2009) also noted the synergistic effects of antibiotics mixtures with the same directional effect. However, the causes of such effects are still not clear.

In the field model experiment, the influence of chernozem pollution by antibiotics on the growth and development of peas was examined. Numerous studies show that manifestations of the toxic effects of antibiotics are very diverse. They are associated with a delay in the growth and development of plants, suppression of seed germination, inhibition of root growth and elevated parts of plants, a violation of the chlorophyll formation, etc. (Dmitrieva, & Semenov, 1965; Lesovoi, 1989). Generative organs of plants are exposed to the negative effects of antibiotics to greater extent than the vegetative ones. Pollution of the soil by antibiotics at a concentration of 500 mg/kg had practically no effect on the morphological indicators of the vegetative organs of peas, while the indicators of the state of the generative organs, including the yield, were reduced by up to 1.5 times.The obtained results confirmed the regularities obtained earlier that generative organs of plants were exposed to greater negative influence of pollutants than the vegetative ones (Boxall *et al.*, 2006).

The IIBS was reduced compared with the control at the first stages of the study (by 8% for tylosin and by 11% for oxytetracycline). At further stages of the study, the difference in the IIBS values in relation to the control and soil samples under peas cultivation, polluted by antibiotics, was not significant. Most likely, such a difference in the IIBS values in soil samples polluted by antibiotics and soil images under peas' cultivation polluted by antibiotics was explained by the inactivation of antibiotics from the soil by the roots of plants. Numerous experimental studies (Stackelberg *et al.*, 2004; Auerbach *et al.,* 2007; Zhang *et al.,* 2009; Li *et al.*, 2010; Gao *et al.*, 2012; Rizzo *et al.*, 2013) showed that the majority of antibiotics were adsorbed by the roots of plants. The rate of antibiotics penetration into plants was determined by their chemical structure. Acidic and neutral antibiotics (chloramphenicol, penicillin) penetrated plant tissues most rapidly. Amphoteric antibiotics such as chlortetracycline and oxytetracycline and antibiotics-bases (neomycin, streptomycin) penetrated plant tissues more slowly.

When comparing the results of laboratory and field modeling of chernozem pollution by antibiotics, it was established that, on the whole, the regularities obtained in laboratory conditions were preserved even in field conditions. Differences between variants polluted by antibiotics in the field and in the laboratory were maintained. At the same time, the rate of recovery of the biological activity of chernozem after pollution in the field was two times higher. This is explained, most likely, by the fact that antibiotics decompose faster in the soil in the field conditions. Some of them are destroyed by sunlight, some are inactivated by soil solution or destroyed by microbes, some are washed away with water, and a large part is adsorbed by soil particles.

Despite the tendency of recovery of biological indicators, after the pollution by antibiotics, both in laboratory and field conditions, they do not completely recover. This indicates the prolonged nature of antibiotics' action and their relative stability in the soil. According to the literature, the rate of antibiotics' destruction in the soil is different. Some of them are inactivated in the soil in the first hours after application, others can be preserved for a long time – several days or even several weeks, depending on the nature of the substance and substrate properties (Braschi *et al.,* 2013). For example, chlortetracycline can persist in the soil without any changes up to 5 months at a concentration of 250 μg/kg or in water at a concentration of 20 mg/kg. The half-life period of chlortetracycline in bottom sediments at fish factories can be 90 to 144 days (Loftin *et al.,* 2008).

The degree of decrease in biological indicators was more pronounced in laboratory conditions than in the field ones. The rate of recovery of the biological activity of chernozem after pollution in the field was two times higher. This is explained, most likely, by the fact that antibiotics are decomposed faster in the field conditions under the influence of climatic factors (light, temperature, etc.); some of them are inactivated by soil solution, some are washed away with water, and a large part is adsorbed by soil particles.

The maximum exposure to antibiotics was manifested in the first 10 days from the moment of chernozem pollution. Then there was a tendency to restoring biological indicators.

Different biological indicators reacted to the pollution by antibiotics to varying degrees. According to the degree of resistance to antibiotics, the investigated microorganisms of chernozem formed the following sequence: bacteria of the genus Azotobacter > micromycetes > amylolytic bacteria > ammonifying bacteria. Enzymes formed the following sequence: peroxidase ≥ polyphenol oxidase > catalase > dehydrogenase > invertase ≥ phosphatase.

Antibiotics have prolonged influence on the biological properties of chernozem. The dynamics of recovery, both in the number of microorganisms and in fermentative activity, is nonlinear.

Conclusion

Antibiotics have prolonged influence on the biological properties of chernozem. The dynamics of recovery, both in the number of microorganisms and in fermentative activity, is nonlinear. Further studies are needed to find the mechanism (mechanisms) of influence of antibiotics on a microbiota and enzymatic activity, for different types of soils as noted in the present study.

Declarations

Data availability

Primary data are presented in tables that accompany the text of the article. This dataset is the result of field and laboratory studies. It has not previously been published and can be used to verify the conclusions presented in this paper.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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Tables

Table 1: Characteristic of the chernozem ordinary (Botanical garden of SFU, Rostov-on-Don) (Guide ..., 2008)

Soil horizon	Soil thickness, cm	pH	Organic matter, %	Catalase activity (mL O2/g/min.)	Dehydrogenase activity (mg triphenylformazan/1g/24 hrs.)	Cation exchange capacity (mEq / 100 g)	Granulometric texture
Ад	0–10	7,7	4,95	8,7	18,7	37,5	heavy clay loam
A	10–25	7,7	3,33	8,3	18,3	35,5	
AB	25–60	8,0	2,98	8,5	9,8	30,1	
BC	60–80	8,2	1,05	5,2	7,8	19,6	
C	80–110	8,3	0,25	4,6	5,0	–	

Table 2: Dynamics of changes in the total number of chernozem bacteria in case of pollution by tylosin, bn/g

10 days				
	Control	10 mg/kg	100 mg/kg	1000 mg/kg
M ±m	11,400,01	10,10,08	6,600,22**	3,900,05***
s	0,02	0,30	0,90	0,20
CV, %	0,31	3,38	13,46	0,21
% of control		89	58	35
60 days				
M ±m	11,720,14	10,590,11	8,630,17*	6,630,13**
s	0,50	0,40	0,70	0,50
CV, %	4,65	4,01	8,03	8,11
% of control		90	74	57
120 days				
M ±m	11,890,04	11,020,05	9,600,07*	9,120,12**
s	0,20	0,20	0,30	0,50
CV, %	1,31	1,67	2,95	5,12
% of control		93	81	77

Note. Significant differences in relation to the control: *p < 0.05; **p < 0.01; ***p <0.001, given n=4.

Table 3: The effect of antibiotics at a concentration of 100 and 600 mg/kg on the enzymatic activity of ordinary chernozem

Days	Control	Benzylpenicillin	Benzylpenicillin + nystatin	Pharmasin	Pharmasin + nystatin
Catalase activity (mL O2/g/min.)					
10	16,43±0,03	11,17±0,04**/10,27±0,05**	11,97±0,04**/10,73±0,14**	12,10±0,04*/11,73±0,04*	8,80±0,08***/5,77±0,08***
60	12,26±0,08	11,42±0,03/11,13±0,01	11,97±0,04/10,73±0,14	11,30±0,05/10,85±0,01	10,47±0,09*/10,16±0,02*
120	11,59±0,13	10,00±0,01*/9,20±0,05*	10,67±0,04/9,83±0,04	10,23±0,06**/8,50±0,05**	9,77±0,04*/9,07±0,11*
Dehydrogenase activity (mg triphenylformazan/1g/24 hrs.)					
10	31,34±0,32	22,60±0,69**/19,30±0,59***	21,50±0,48**/17,38±0,06***	24,00±0,47**/16,47±0,19***	21,90±0,85**/9,65±0,33***
60	33,65±0,27	32,30±0,08/29,42±0,26	29,00±0,55*/24,58±0,22**	31,50±0,15/28,66±0,13	27,40±0,30*/23,56±0,26**
120	26,30±0,02	22,68±0,01*/21,90±0,01*	24,93±0,01/24,11±0,02	22,19±0,01*/ 21,37±0,01*	24,50±,01/24,29±0,02
Phosphatase activity (mg P2O5/10g/1h.)					
10	3,60±0,01	1,97±0,01***/1,07±0,01***	2,29±0,01**/1,4±0,01***	2,50±0,01**/1,14±0,01***	2,76±0,01*/1,90±0,01***
60	3,11±0,02	2,83±0,01/1,68±0,02***	2,90±0,02/1,82±0,02***	2,69±0,03*/1,68±0,02***	2,76±0,01*/1,90±0,02***
120	3,14±0,01	2,15±0,02**/1,54±0,0***	2,69±0,01*/1,72±0,02***	2,43±0,02**/1,36±0,01***	2,18±0,01**/1,65±0,02***
Invertase activity (mg glucose/1g/24 hrs.)					
10	30,06±1,4	22,10±0,05**/15,23±0,5***	28,40±1,3/23,42±0,5*	22,60±0,06**/8,26±0,1***	29,80±1,2/23,50±0,4*
60	30,96±0,08	25,00±0,1*/23,31±0,08*	28,40±1,3/23,42±0,08*	24,70±0,2*/22,73±0,08*	29,80±1,2/23,50±0,08*
120	25,34±0,04	24,63±0,1/22,89±0,4	24,02±0,1/22,31±0,2	23,69±0,4/21,46±0,3*	24,35±0,2/22,29±0,2

Note. Significant differences in relation to the control: *p < 0.05; **p < 0.01; ***p < 0.001, given n = 4

Table 4: The influence of chernozem pollution by antibiotics on the growth and development of peas (Botanical Garden of the Southern Federal University, 2014)

No.	Indicators	Antibiotics (500 mg/kg)					
		Control		Tylosin		Oxytetracycline	
		M± m	*CV, %*	*M± m*	*CV, %*	*M± m*	*CV, %*
1	Common phytomass (with pods), g	350±1.76	1.51	213±8.26	17.48	193±8.55	18.86
2	Total number of pods, pcs.	107±2.34	6.54	95±4.12	27.64	91±6.88	22.77
3	Total weight of pods, g	84.05±1.94	6.92	73.30±0.94	16.90	75.95±6.57	25.96
4	Weight of pods without peas, g	17.23±0.62	10.8	16.88±1.1	10.58	16.48±1.28	23.39
5	Average length of pods, cm	4.73±0.51	3.23	4.61±0.60	6.12	4.57±0.40	2.66
6	Total weight of peas, g	69.82±0.01	1.00	56.42±0.30	1.00	59.47±0.01	1.00
7	Weight of 100 peas, g	19.92±0.21	3.22	19.33±0.17	4.68	20.01±0.55	8.19
8	Average number of peas in pods, pcs.	6.00		6.00		6.00	
9	Yield, dt/ha	12.83		11.02		10.89	

Figures

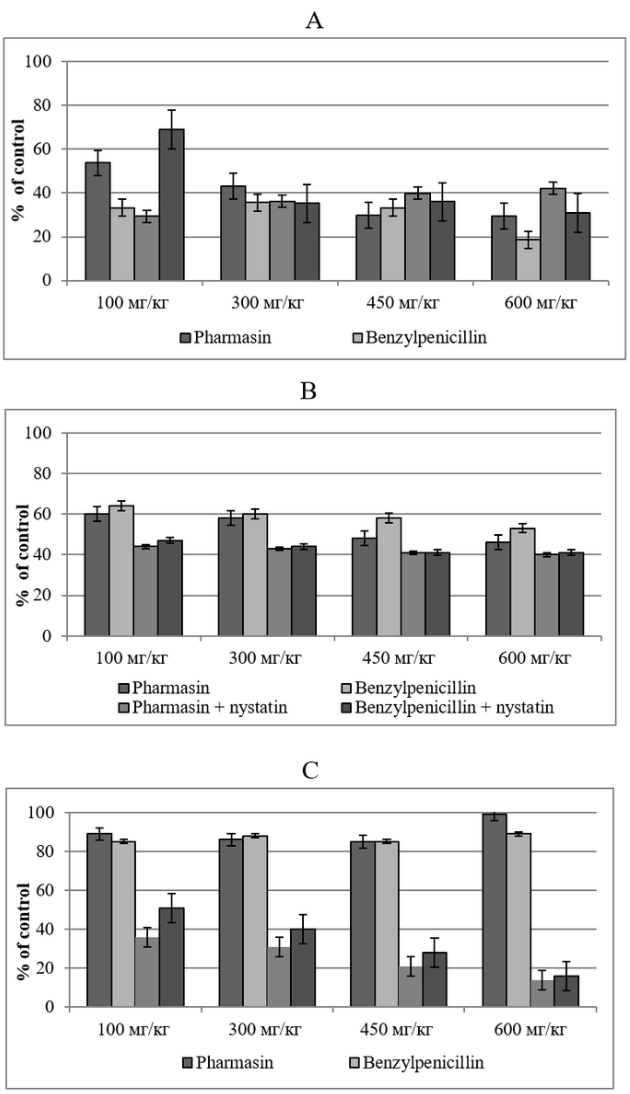

Figure 1

Change in the number of the main groups of microorganisms of chernozem in cases of pollution by antibiotics (A – ammonifying bacteria, B – amylolytic bacteria, C – micromycetes), % from the control

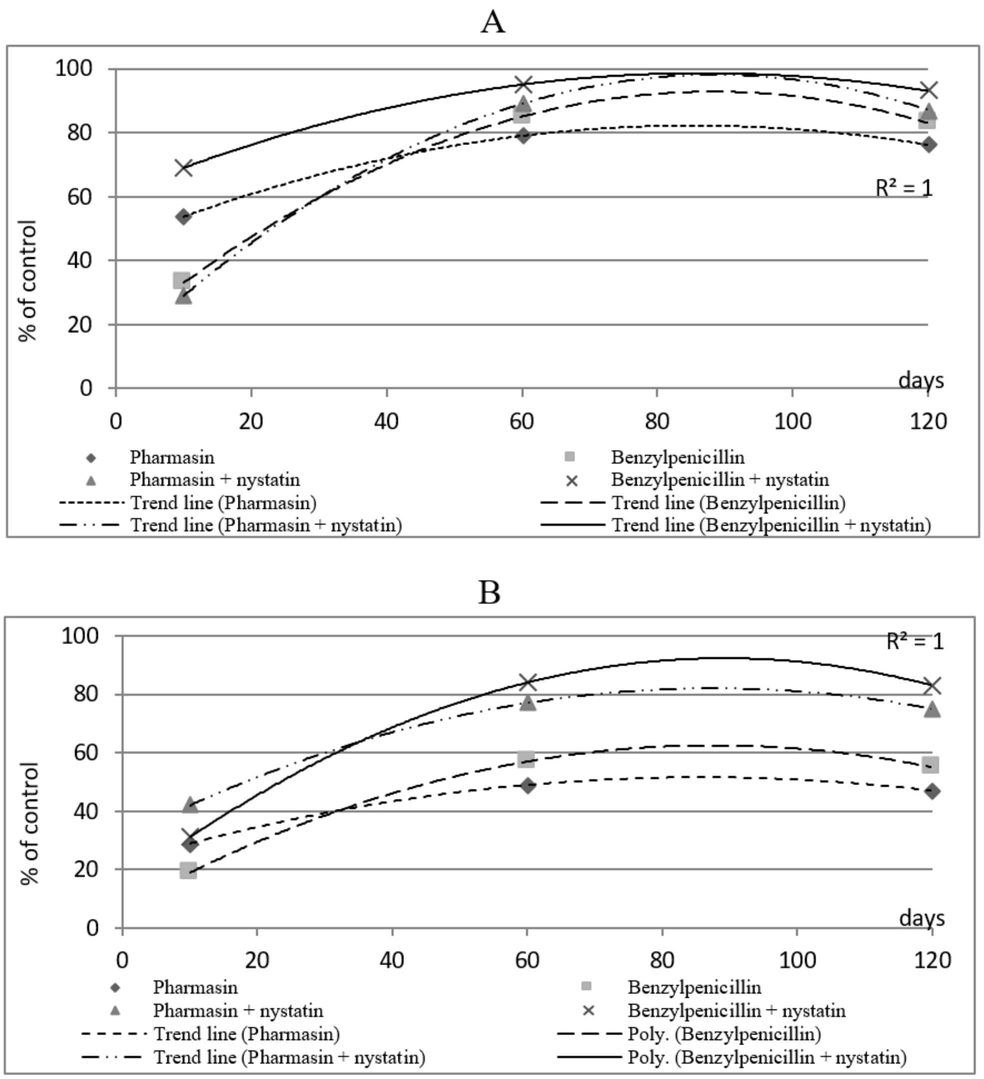

Figure 2

Dynamics of the number of ammonifying bacteria of chernozem when polluted by antibiotics (A – 100 mg/kg, B – 600 mg/kg)

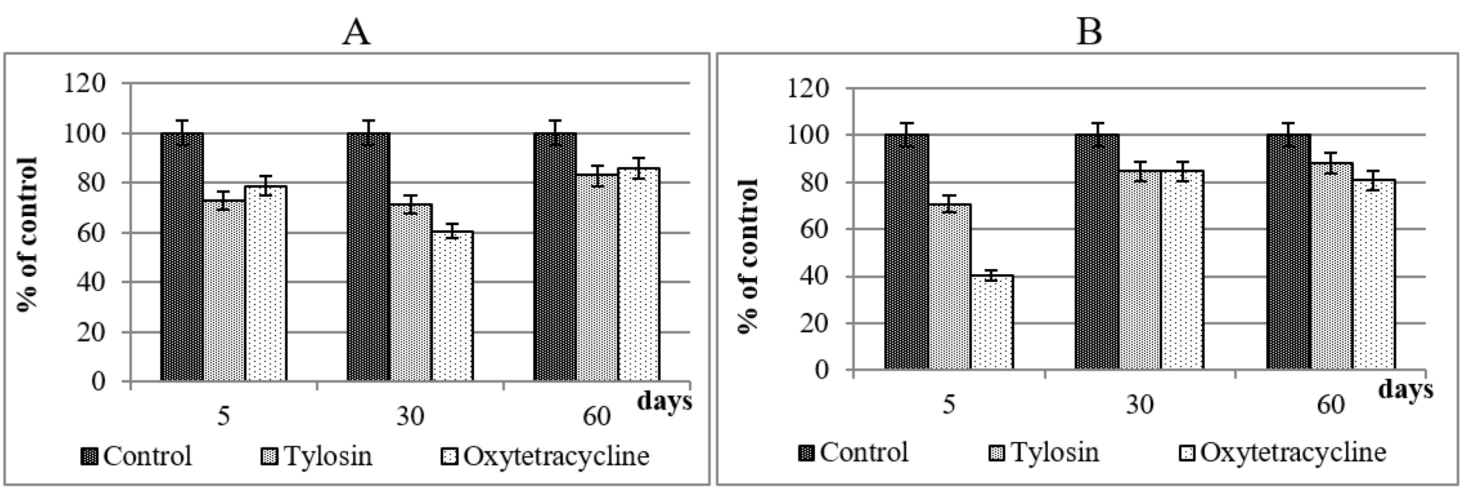

Figure 3

The change in the number of chernozem microorganisms when polluted by antibiotics in the field conditions (500 mg/kg) (A – ammonifying, B – amylolytic bacteria)

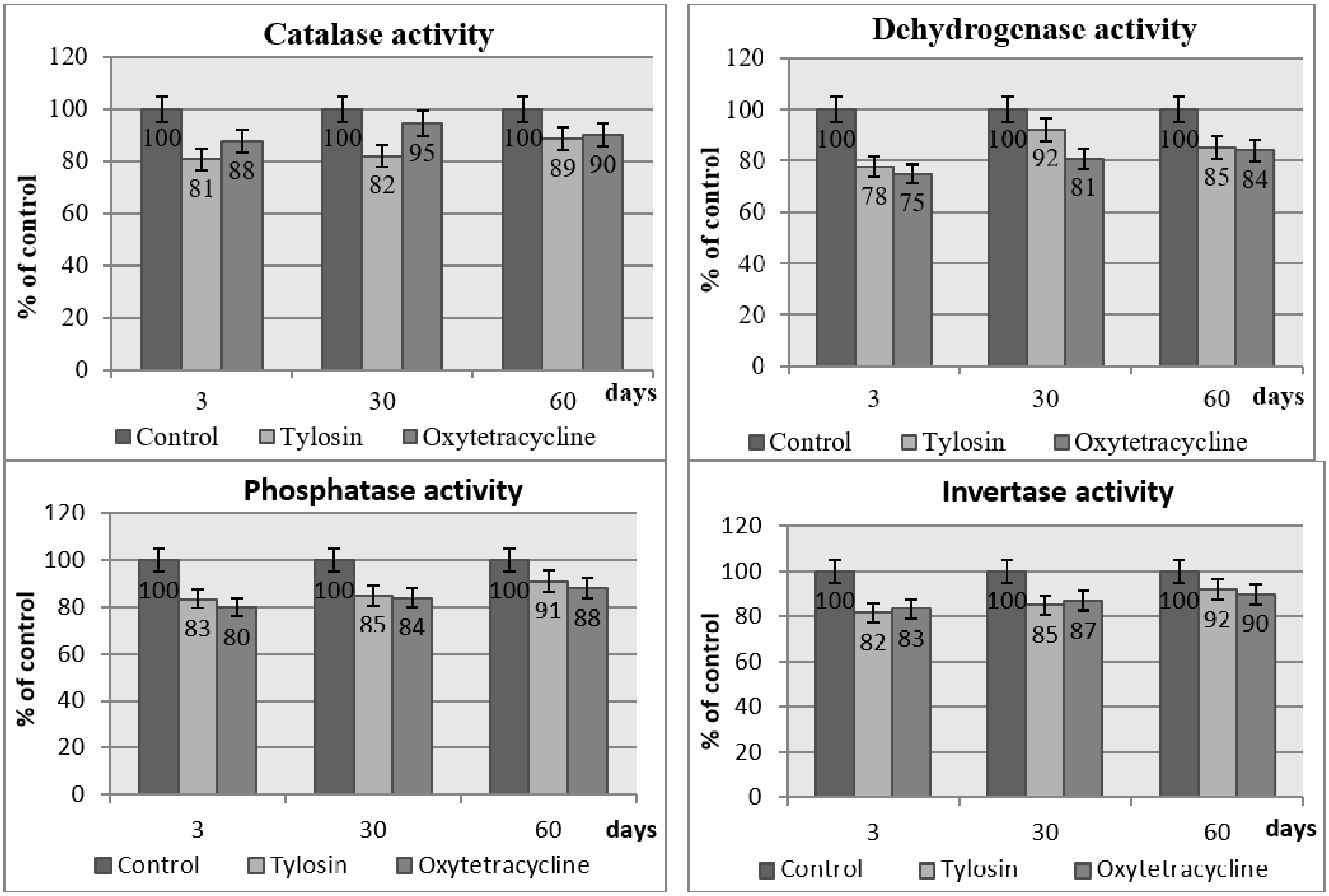

Figure 4

Dynamics of changes in the enzymatic activity of chernozem after the pollution by antibiotics in the field conditions (500 mg/kg)

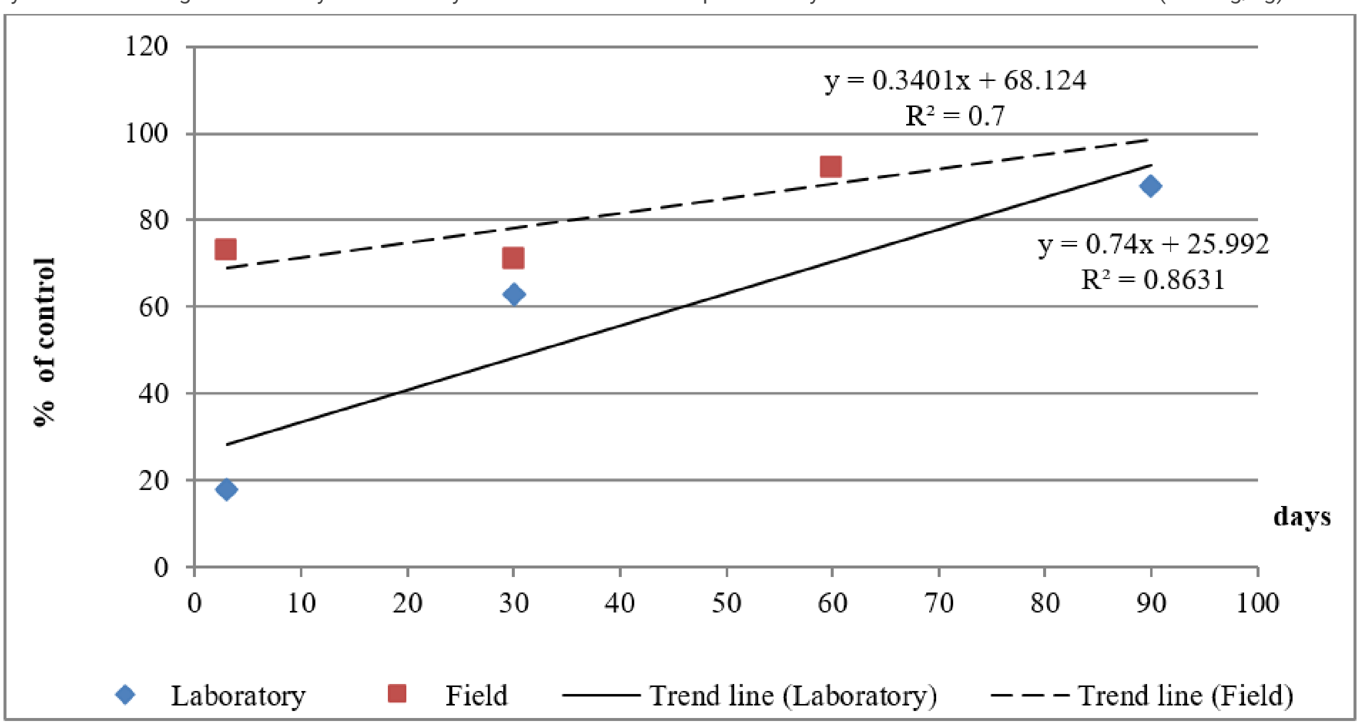

Figure 5

Comparison of the effect of pollution by tylosin antibiotic on the number of ammonifying bacteria of ordinary chernozem in laboratory conditions and in the field

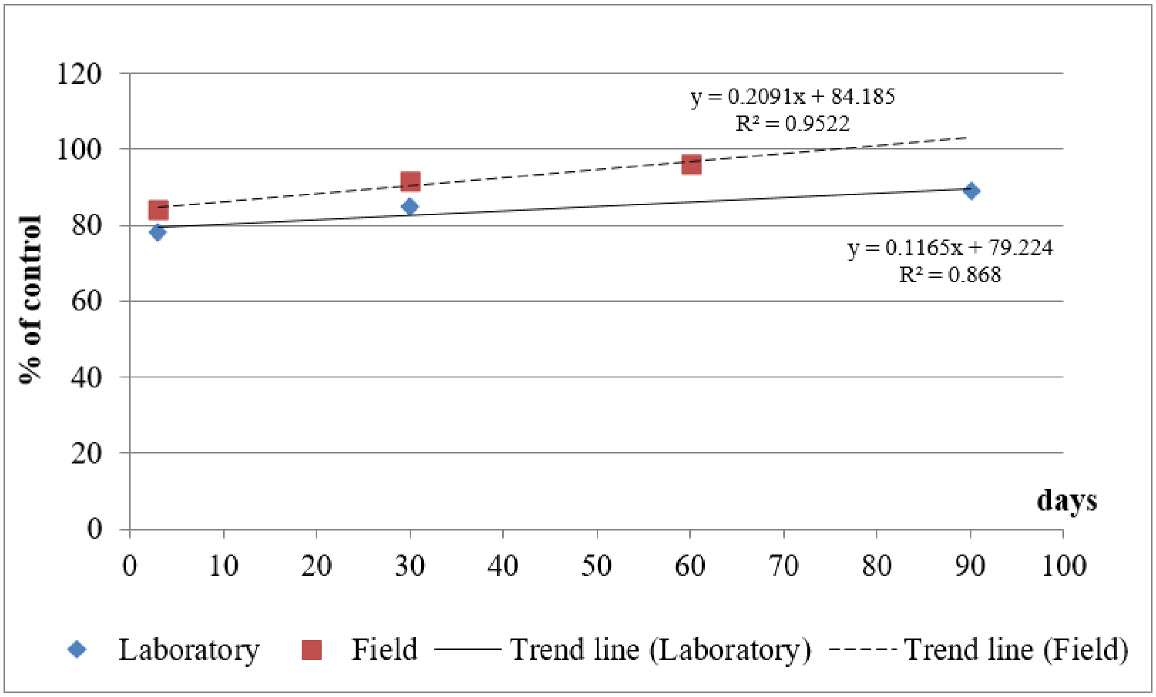

Figure 6

Changing IIBS of ordinary chernozem polluted by antibiotic tylosin in laboratory conditions and in the field