

# Influence of Antibiotic Contamination on Functioning of Microbiocenosis of Ordinary Chernozem

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## Abstract

**Objective:** Wide-scale application of numerous antibiotics with various chemical compositions and spectra in medicine, agriculture, and food industry is accompanied by violation of microbial community in various ecosystems. Therefore, the resistance of microbial community of soils to contamination with antibiotics widely applied in medicine and agriculture (tylosin and oxytetracycline) has been assessed. Antibiotic contamination leads to decrease in the count of main ecotrophic groups of microorganisms, variation of their ratio, and structure of microbial community in total. **Materials and Methods:** Maximum impact of antibiotics was exposed in the 1<sup>st</sup> day after contamination of chernozem (3 days). Later (30 and 90 days), the trend of recovery of biological performances was observed, and dynamics of recovery of both microorganisms and enzyme activity was non-linear. **Results:** In terms of resistance to antibiotics, the considered microorganisms could be ranged as follows: Micromycetes > p. *Azotobacter* bacteria > amylolytic bacteria > ammonifiers. Comparison of laboratory and field simulation of antibiotic contamination of chernozem revealed that the rate of deterioration of biological performances was more pronounced under laboratory conditions in comparison with the field. **Conclusion:** The recovery rate of chernozem biological activity after field contamination was higher more than by 2 times. Probably, this could be attributed to the fact that in the field environment antibiotics were decomposed more rapidly under climatic impacts.

**Key words:** Contamination, ordinary chernozem, oxytetracycline, soil microorganisms, tylosin`

## INTRODUCTION

Functioning of soil microbiota is the most complex and important aspect of soil diagnostics. Microorganisms as bioindicators of water systems are sufficiently widely applied in hygiene and water management; only initial steps are made in this direction concerning soil systems. Activity of soil microorganisms is a good diagnostic index of soil fertility and its change as a consequence of impact of various anthropogenic factors.

Nowadays, numerous biologically active substances are used in agriculture and medicine, and their production increases significantly year by year. Currently, variation of biological properties of Russian southern soils is studied in details with regard to contamination with oil and gasoline, heavy metals, metalloids,<sup>[1-5]</sup> and pesticides.<sup>[6]</sup> However, contrary to pesticides, which have been used in agriculture for years, antibiotics did not attract attention as potential

environmental contaminants. Taking into consideration intensification of their use in all fields of agriculture, particular attention is paid to the aspect of antibiotic contamination of natural ecosystems. The data by Research Techart Company (2013) evidence that about 3.5 thousand ton of antibiotics are used in Russia every year, including 19% used as growth stimulators and 22% as preventive measures. According to WTO forecast, the use of antibiotics in Russia will increase every year by about 35–40%.

Resistance of microorganisms to various classes of antibiotics is one of the most urgent challenges of our times.

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Its occurrence and rapid spreading create hazardous situation not only in medicine but also in veterinary, cattle breeding, horticulture, and aquaculture.

Antibiotics entry into soil mainly due to the use of manure<sup>[7,8]</sup> and wastewaters<sup>[9,10]</sup> on agricultural soils as fertilizers. In the course of time, various contents of antibiotics are detected in wastewaters, soils, ground, and drinking water.<sup>[11-13]</sup> Antibiotics of tetracycline family are detected *in situ* from trace amounts to 900 mg/kg<sup>[14]</sup> and of macrolide family up to 800 mg/kg.<sup>[15]</sup>

## MATERIALS AND METHODS

Field simulations were carried out on fallow lands of protected steppe of the Botanic Garden of the Southern Federal University on the basis of laboratory experimental results by Yu, et al.<sup>[16-19]</sup> Ordinary chernozem of southern European facie (north Azov area) in the garden territory was the dominating soil. These soils were characterized by high amount and variety of microorganisms and their high activity. In field simulations, the plots were arranged according to common procedure of field tests.<sup>[20]</sup> Microbiocenosis variation dynamics were analyzed after 3, 30, and 60 days from the date of antibiotic contamination. Antibiotics (oxytetracycline and tylosin) were added into soil in the concentration of 500 mg/kg soil.

Integrated study of microbiological properties of ordinary chernozem included determination of count of viable microorganisms of various ecotrophic groups by inoculation of respective dilutions (2;3;5) onto solid nutrient medium: Meat peptone agar was used for amount determination of ammonifiers, starch, and ammonia agar - for determination of amylolytic bacteria, Czapek's agar - for determination of abundance of micromycetes. Abundance of *p. Azotobacter* was determined by mud balls fouling on Ashby agar. In addition, total bacterial count was determined by luminescence microscopy with sample coloration by acridine orange.<sup>[21]</sup>

Statistic processing of experimental results was performed using Statistica 10.0 and Excel.

## RESULTS AND DISCUSSION

Soil acidity is an important chemical index influencing activity of soil microbiota.<sup>[22]</sup> On the basis of field simulation, it was established that antibacterial antibiotics led to slight pH displacement to acidic region and fungicidal antibiotics to basic region. Probably, soil acidity varied either due to direct chemical action of antibiotics decomposition (metabolites) or due to transformation under the impact of microbial community, and hence, its biochemical impact on soil.

Antibiotic contamination of chernozem led to variation in the count of main microorganisms. The considered groups of microorganisms differed in terms of their resistance to added antibiotics. The highest suppressive impact on the count of ammonifiers (by 20–60% of reference,  $P < 0.05$ ,  $n = 6$ ) was that of oxytetracycline at all stages of tests. Statistically confident differences in the count of ammonifiers on the 3<sup>rd</sup> and 30<sup>th</sup> days were not found. Recovery of count of this bacteria group was not observed on the 60<sup>th</sup> day of tests.

Amylolytic bacteria were more resistant to the considered antibiotics than ammonifiers. On the 3<sup>rd</sup> day of tests, maximum decrease in bacteria count was observed (60% of reference,  $P < 0.001$ ). Similar to ammonifiers, the highest inhibiting action was exerted by oxytetracycline. At subsequent stages of exposure, no confident variations of amylolytic bacteria count were found, and there was a trend of count recovery to reference values.

A prolonged pattern of impact of antibiotics on soil microorganisms evidenced their relative resistance in soil. Stone et al.<sup>[23]</sup> detected active metabolites of tylosin after 150 days from the date of its addition to soil. Loke et al.<sup>[24]</sup> demonstrated that at pH below 7.4 strong ionic bonds were generated between protonated tylosin and anionic soil components, which evidenced high sorption of tylosin in soil. Positive correlation of sorption of tylosin and oxytetracycline with humus content, pH, and capacity of ionic exchange of soils was established.<sup>[25]</sup> High capability to sorption of tylosin was observed in humus rich soils rather than in sandy soils.<sup>[26]</sup>

In the scope of field simulation, antibiotics actually did not have influence on micromycete count of chernozem, which was attributed to the essence of the considered antibiotics, tylosin and oxytetracycline were efficient only with regard to bacteria. On the 30<sup>th</sup> and 60<sup>th</sup> days of tests, no variations in micromycete count were observed.

Variation in abundance of *p. Azotobacter* bacteria was observed only on the 3<sup>rd</sup> day of tests (decrease by 30–40% of reference on impact of tylosin and by 50–60% on impact of oxytetracycline, respectively), and at other stages, no confident variations in bacteria abundance were observed. Herewith, nitrogen-fixing bacteria were more sensitive to contaminations with other impurities (heavy metals, oil, and ionizing radiation) in comparison with other bacterial groups.<sup>[27]</sup>

By means of luminescence microscopy, decrease in total bacterial count was established on antibiotic contamination of chernozem. Close correlation was determined between antibiotics' concentration and bacterial count ( $r = -0.85$ ). The highest decrease in total bacterial count occurred at the first stage after contamination. Then, the trend of recovery was observed. Despite the observed trend even after 60 days from the date of contamination, the decrease in bacterial count equaled to 20% of reference.

Therefore, in terms of resistance to antibiotics, the considered microorganisms were classified as follows: Micromycetes > *p. Azotobacter* bacteria > amyolytic bacteria > ammonifiers. Microbiological indices were of non-linear nature. In terms of recovery rate, the microorganisms of ordinary chernozem were classified as follows: *p. Azotobacter* bacteria > micromycetes > amyolytic bacteria > ammonifiers.

The results of laboratory and field tests of antibiotic contamination of chernozem were compared. At the first stage of exposure, the count of main groups of the considered microorganisms sharply decreased, only groups of microorganisms resistant to antibiotics remained. Resistant microorganisms were detected in various soils, marine and river deposits, excavation of abandoned mines, waters of seas, rivers and lakes, underground waters, irrigation channels, water drainage from irrigation fields, settling ponds in agricultural farms, and so on.<sup>[28]</sup> As a consequence, new genes resistant to antibiotics were revealed, not registered in the case with the so-called clinical isolates of pathogenic microorganisms.<sup>[29]</sup> Therefore, microorganisms resistant to antibiotics nearly always exist in the composition of various natural microbial communities.

To the highest extent, antibiotics inhibit growth and development of ammonifiers [Figure 1]. Amyolytic bacteria exhibited relative resistance both under laboratory conditions and in the field environment. Tylosin did not exert confident suppressive impact on the count of micromycetes of ordinary chernozem, which was related with its chemical structure. At all stages of exposure, minor variations of this group of microorganisms were observed.

While studying recovery of soil microbiocenosis, it was established that the impact of antibiotics was prolonged; however, there was a trend to count recovery of microbiocenosis under laboratory conditions on the 90<sup>th</sup> day and under field

conditions on the 60<sup>th</sup> day. Probably, soil microorganisms were readily adapted exhibiting resistance to added antibiotics.

Increase in the count of antibiotic resistance genes in environment as a consequence of *in situ* anthropogenic impact occurred not only in local ecotope but also it led to their wide spreading in the given area. In microbial communities of various areas in the vicinity of agricultural farms, where antibiotics were used, a significant increase in antibiotic resistance was observed. For instance, tetracycline resistance genes had been found not only in farms but also in water of local rivers, river deposits, irrigation channels, and even in drinking water.<sup>[30]</sup>

## CONCLUSIONS

Negative influence of antibiotic contamination on microbial community of ordinary chernozem has been detected. In terms of resistance to antibiotics, the considered microorganisms can be ranged as follows: *p. Azotobacter* bacteria > micromycetes > amyolytic bacteria > ammonifiers. The impact of antibiotics on soil microbiocenosis is prolonged. Dynamics of recovery of microorganisms is non-linear. In terms of recovery rate, microorganisms of ordinary chernozem are classified as follows: *p. Azotobacter* bacteria > micromycetes > amyolytic bacteria > ammonifiers. The most informative of the considered microbiological indices on antibiotic contamination is ammonifier count. The abundance of *p. Azotobacter* bacteria on antibiotic contamination is non-informative.

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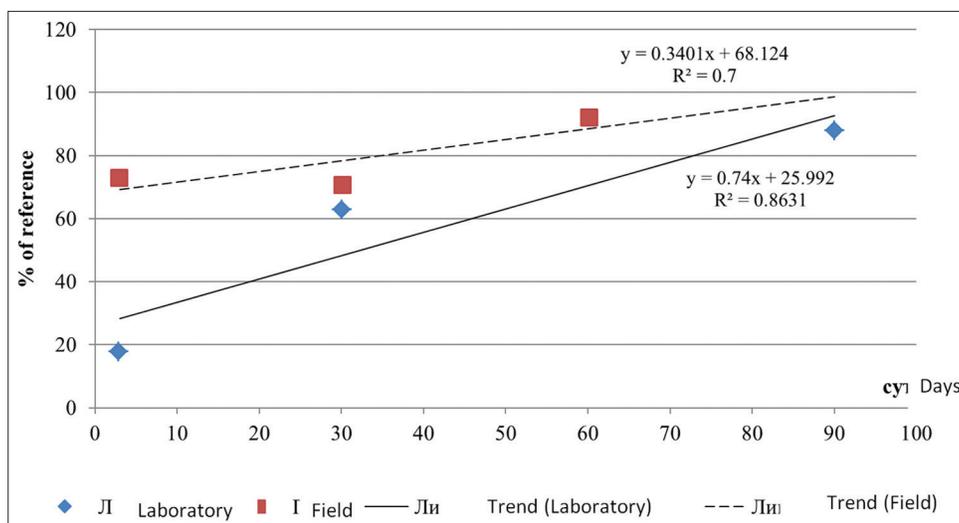


Figure 1: Comparison of laboratory and field tests of contamination with tylosin on count of ammonifiers in chernozem

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