ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Assessment of Chernozem Environmental and Agricultural Capabilities' Resistance to Antibiotic Contamination

Yuliya Viktorovna Akimenko, Kamil Shagidullovich Kazeev, and Sergey Il'ich Kolesnikov

Southern Federal University, Russia, 344090, Rostov-on-Don, Stachki, 194/1

Abstract

This article presents laboratory simulations of ordinary chernozem environmental and agricultural capabilities' resistance to antibiotic contamination on the basis of integral index of biological state of soil (IIBS). The experimental results have revealed negative impact of antibiotic contamination of ordinary chernozem in terms of main biological performances' variation with violation of soil environmental capabilities (decrease in IIBS by 10-25% in comparison with reference). Antibiotics' influence extent is determined by their essence and impact duration. Bactericidal antibiotics exert higher suppressive action on the considered performances than bacteriostatic ones. In terms of inhibition of biological performances antibiotics can be ranged as follows: ampicillin ≥ streptomycin > tylosin > tromexin > aliseryl. The most informative among the studied biological performances is the ammonifier count and the activity of dehydrogenase. The least informative is the activity of catalase. Abundance index of *p. Azotobacter* upon antibiotic contamination is not informative.

Key words: contamination, antibiotics, ordinary chernozem, environmental capabilities of soil, soil microorganisms, enzyme activity.

INTRODUCTION

Extensive data on the influence of various anthropogenic impacts on soils state are available in Russian and foreign publications. Most studies are devoted to aspects of soils' chemical contamination and preservation of soils' agricultural and environmental capabilities aiming at provision of food and health safety of humanity. At present variations of biological properties of various Russian soils are sufficiently deeply studied upon contamination with heavy metals, oil and petroleum products [1, 2], pesticides [3, 4]. However, contrary to pesticides, which are 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, topsoil in particular.

Nowadays scientific concern is concentrated, on the one hand, on study of routes of entry, distribution and behavior of antibiotics in environment, and on the other hand, on their impact on other organisms. Long time impact of antibiotics can increase selection of steady bacterial species. Resistance of microorganisms to antibiotics in waste and surface water [5, 6], soil [7] has been detected.

The main sources of entry of antibiotics into environment are pharmaceutical products, veterinary, and human medicine. At present in many countries numerous antibiotics are applied in cattle and plant breeding both for cattle growth stimulation and as preventive measures. The used antibiotics migrate along food chains accumulating in plants, in particular in vegetables and fruits [7].

Antibiotics entry into soil due to the use of manure and waste waters as fertilizers on agricultural soils [8]. In the course of time various contents of antibiotics are detected in waste waters, soils, ground and drinking water. Antibiotics of tetracycline family are detected in situ from trace amounts to 900 mg/kg, and of macrolide family – up to 800 mg/kg [9].

According to WTO forecast, the use of antibiotics in Russia will increase every year by about 35–40%. In most European countries it is prohibited to apply antibiotics as feed supplement and as preventive measures. In Russia there are no regulatory specifications on content and detection of antibiotics in natural environment, feed antibiotics are not prohibited.

Therefore, it seems to be promising to carry out diagnostics, monitoring and standardization of antibiotics impact on soil in terms of violation of environmental and agricultural capabilities performed by soil in natural and agricultural ecosystems. Violation of soil agricultural and environmental capabilities occurs in certain sequence depending on the rate of anthropogenic influence. Usually, at first informative capabilities are violated, then biochemical, physicochemical, chemical, integral, and, finally, physical capabilities of soil. Criterion of violation extent of these or those soil capabilities is the integral index of biological state of soil (IIBS), which is determined on the basis of set of the most informative biological properties, which response first to anthropogenic impact. When the integral index decreases, various soil capabilities are violated.

The work is aimed at assessment of soil environmental and agricultural capabilities' resistance to antibiotic contamination on the basis of ordinary chernozem biological performances.

MATERIALS AND METHODS

The subject of this study is microbiocenosis of ordinary chernozem, southern European facie, carbonaceous, slightly humic, loamy, on fluvic loess loams. Ordinary chernozem soils (Voronic Chernozemes Pachic) occupy vast areas of Azov Kuban plain in Krasnodar krai and south of Rostov oblast, according to new classification:

migrating segregating chernozem soils [10]. Chernozem soils are selected as subject of study, since they are characterized by high amount and variety of microorganisms and their high activity. In addition, chernozem zone is an important arable region of Russia. More than half of arable soils are presented by chernozem, where grain crops, oil and industrial crops are cultivated. These are regions characterized by widely developed cattle breeding and horticulture. Soils were sampled for simulations in Botanic garden of the Southern Federal University, Rostov-on-Don (arable layer: 0-25 cm).

In order to solve the predetermined targets we performed a series of simulating laboratory tests. The influence of medical antibiotics - ampicillin, streptomycin - and veterinary antibiotics - tylosin, tromexin, aliseryl - in concentration of 500 mg/kg on biological properties of ordinary chernozem was studied. This concentration was selected on the basis of published data according to residual amounts of antibiotics, detected in environment [11] and previously performed tests [12, 13]. Antibiotics were added into soil in the form of solutions. The studies were performed on the 3rd, 30th, and 90th day after contamination. All soil samples were incubated in dark place at 20–25°C and optimum moisture content (60% of field water capacity). Soil without antibiotic contamination served as reference. Analytical measurements of biological properties of chernozem were performed three-fold in order to study microbiological properties of soils and nine-fold in order to study biochemical properties.

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 (MPA) was used for amount determination of ammonifiers, starch and ammonia agar (SAA) - 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. Biochemical properties of soil were studied by the following methods: determination of catalase activity (Galstyan, 1978), determination of dehydrogenase activity (Galstyan, 1982). General regularities of antibiotic contamination influence on biological state of ordinary chernozem were evaluated by integral index of biological state of soil (IIBS). IIBS was determined on the basis of the most informative biological performances [14].

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

RESULTS AND DISCUSSION

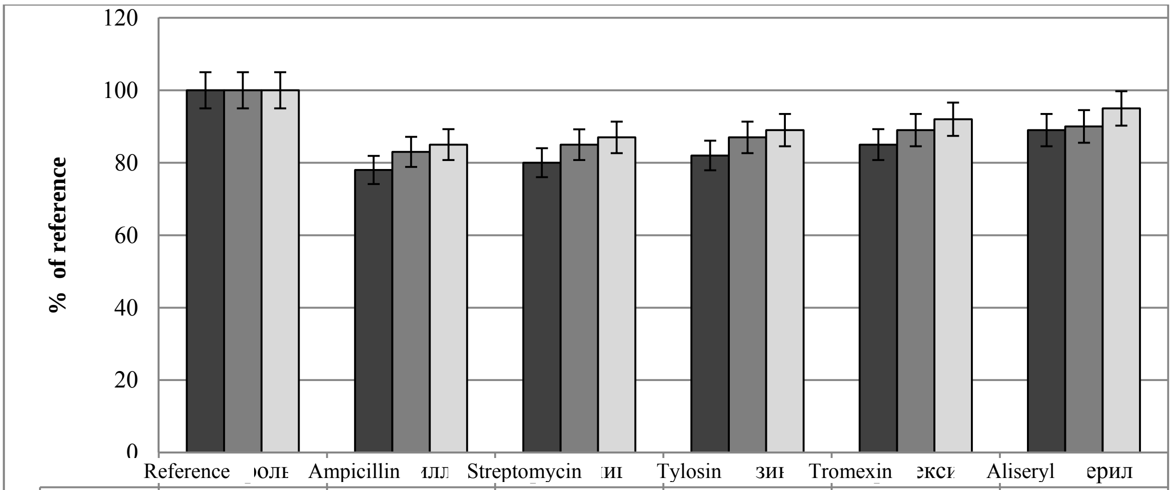
Analysis of the experimental results demonstrated that antibiotic contamination of chernozem exerted inhibiting impact on viability of ammonifiers, similar results were obtained in another works [15]. Ampicillin, streptomycin and tylosin caused decrease in ammonifier amount more than by 40% of reference. The highest inhibiting impact was that of ampicillin (decrease by 50-60 % of reference, $p<0.001$) in initial periods from the date of contamination (3 days). At subsequent stages of study (30, 90 days) insignificant trend to recovery of ammonifier amount was observed, but even in 90 days after contamination recovery to the reference level was not achieved, the difference between bacteria count in comparison with reference was 20-30% for ampicillin, streptomycin, and tylosin. In samples contaminated with tromexin and aliseryl no confidential difference in ammonifier amount was detected after 90 days.

Upon antibiotic contamination of chernozem confidential decrease in amylolytic bacteria count was observed at the first stage after contamination (3 days). The highest inhibiting impact on abundance of amylolytic bacteria was exerted by ampicillin and streptomycin (decrease by 50-60% and 40-50%, respectively, $p<0.001$). Soil contamination with tromexin and aliseryl did not result in confidential variations of abundance of amylolytic bacteria after 3 and 90 days from the date of contamination. At the first stage of studies (3 days) upon contamination with these antibiotics the count of amylolytic bacteria decreased by 10-15% in comparison with reference. In another works it was established that sulfonamide antibiotics, tetracycline, trimethoprim [16] in concentrations of 300–500 mg/kg exerted significant inhibiting impact on count of amylolytic bacteria. At subsequent stages of the studies (30, 90 days) the count of amylolytic bacteria was recovered, especially in soil samples contaminated with tromexin and aliseryl.

As expected, antibacterial antibiotics did not cause statistically confidential decrease in abundance of micromycetes. In soil samples contaminated with ampicillin, streptomycin, and tylosin after 3 and 90 days from the date of contamination abundance of micromycetes exceeded reference by 10-20% ($p<0.05$).

P. *Azotobacter* bacteria were the most resistant to antibiotic contamination. Abundance of bacteria was varied only after 3 days from the date of antibiotic contamination (decrease by 20% of reference upon contamination with ampicillin, by 40% - upon contamination with streptomycin, by 18% - upon contamination with tylosin). Tromexin and aliseryl did not exert confidential inhibiting impact on abundance of bacteria during overall period of studies. Herewith, in studies by other researchers *p. Azotobacter* bacteria were more sensitive to various types of contamination, for instance, with oil and petroleum products, heavy metals, ionizing radiation, in comparison with other groups of soil microorganisms [1, 17].

We studied the influence of antibiotics on activity of enzymes of two oxidase classes (catalase, dehydrogenase). Contamination of ordinary chernozem with antibiotics led to decrease in activity of all considered enzymes. Concerning enzymes of oxidase class, activity of dehydrogenase decreased more in comparison with that of catalase. While studying the influence of antibiotics (ampicillin, streptomycin, tylosin) in concentration of 500 mg/kg, it was established that activity of catalase decreased by 10–15% upon entry of antibiotics. At subsequent stages there was a trend of recovery of enzyme activity. Among antibiotics, tylosin was the most efficient with regard to dehydrogenase.



	Reference	Ampicillin	Streptomycin	Tylosin	Tromexin	Aliseryl
days :уток	100	78	80	82	85	89
days суток	100	83	85	87	89	90
days :уток	100	85	87	89	92	95

Figure 1. IIBS variation dynamics of ordinary chernozem upon antibiotic contamination (500 mg/kg).

Criterion of violation extent of these or those soil capabilities is the IIBS determined on the basis of set of the most informative biological properties, which response first to anthropogenic impact. When the integral index decreased, various soil capabilities were violated. Simulation results of chernozem antibiotic contamination demonstrated that this contamination resulted in decrease in IIBS (see Figure 1). The highest decrease (by 10-25%) in IIBS was observed at the first stage from the date of contamination (3 days), which evidenced violation not only of soil environmental capabilities, but also of biochemical ones. With increase in incubation times of contaminated samples, IIBS increased. However, despite the observed trend to recovery, the obtained data evidenced violation of soil environmental capabilities even after 90 days from the date of chernozem antibiotic contamination.

CONCLUSION

1. Negative impact of ordinary chernozem antibiotic contamination has been detected in terms of main biological performances' variation, evidencing violation of soil environmental capabilities (decrease in IIBS by 10-25% in comparison with reference).
2. The extent of antibiotics' influence is determined by their essence and impact duration. Bactericidal antibiotics exert higher suppressive action on the considered performances than bacteriostatic ones. In terms of inhibition of biological performances antibiotics can be ranged as follows: ampicillin ≥ streptomycin > tylosin > tromexin > aliseryl.
3. The most informative among the studied biological performances is the ammonifier count and the activity of dehydrogenase. The least informative is the activity of catalase. Abundance index of *p. Azotobacter* upon antibiotic contamination is not informative.
4. Maximum impact of antibiotics is exposed in the first days after chernozem contamination (3 days). Later (30, 90 days) the trend of recovery of biological performances is observed. However, complete recovery of biological performances (to reference point) is not observed at the 90th day after contamination. Dynamics of recovery of both microorganisms and enzyme activity is non-linear.

ACKNOWLEDGMENTS

This work was supported by the grants of the President of the Russian Federation (МК 326.2017.11, НШ-9072.2016.11) and the Ministry of education and science of the Russian Federation (5.5735.2017/8.9).

REFERENCES

1. Kolesnikov S.I., Kuzina A.A., Kazeev K.Sh., Evstegneeva N.A., Akimenko Yu.V. Assessment of resistance of brown forest sour soils of the black sea coast of the Caucasus to the chemical pollution. Ecology, Environment and Conservation, 2016, 22 (3): 519-523.
2. Kolesnikov S.I., Kazeev K.Sh., Tatlok D.R. and Akimenko Yu.V. Biological Condition of the Brown Forest Soils of the Western Caucasus at Pollution by Cadmium, Zinc, Molybdenum and Selenium. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2016, 7(5): 684-689.
3. Kazeev K.Sh., Loseva E.S., Borovikova L.G., and Kolesnikov S.I. Vliyanie zagryazneniya sovremennymi pestitsidami na biologicheskuyu aktivnost` chernozema obyknovennogo [Influence of pesticide contamination on biological activity of chernozem]. Agrokhimiya, 2010, 11: 39–44.
4. Akimenko Yu.V., Kazeev K.Sh., and Kolesnikov S.I. Izmenenie biohimicheskikh svoistv chernozema obyknovennogo pri zagryaznenii biotsidami [Variation of biochemical properties of chernozem upon biocide contamination]. Agrokhimiya, 2015, 3: 81-87.
5. Joy S.R. et al. Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. Environ. Sci. Technol, 2013, 47: 12081–12088.
6. Heuer H. et al. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr. Opin. Microbiol., 2011, 14: 236–243
7. Radjenovic´ J., Petrovic´ M., Barceló D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43, 831–841.

8. Kemper N. Veterinary antibiotics in the aquatic and terrestrial environment: a review. Ecological Indicators. 2008, 8: 1–13.
9. Yang J.–F., Ying G.–G., Zhao J.–L., Tao R., Su H.–C. and Liu Y.–S. Spatial and seasonal distribution of selected antibiotics in surface waters of the Pearl Rivers, China. Environ. Sci. Health, 2011, 46: 272.
10. Shishov, L.L., Tonkonogov, V.D. and Lebedeva, I.I. (2004). Klassifikatsiya i diagnostika pochv Rossii [Classification and diagnostics of Russian soils]. Smolensk: Oikumena, 2004.
11. Sarmah A.K. et al. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere, 2006: 65: 725–759.
12. Akimenko Yu.V., Kazeev K.Sh. and Kolesnikov S.I. The Impact of Antibiotics (Benzylpenicillin, and Nystatin) on the Biological Properties of Ordinary Chernozems. Eurasian Soil Science, 2014, 47(9): 910–916.
13. Akimenko Yu.V., Kolesnikov S.I. and Kazeev K.Sh. Environmental impact assessment of tylosin and nystatin on biological properties of chernozem. 15th International Multidisciplinary Scientific GeoConference SGEM 2015, SGEM2015 Conference Proceedings, June 18-24, 2015, 3(2): 131-138
18.
14. Kazeev, K. Sh., Kolesnikov, S. I., Akimenko, Iu. V. and Dadenko, E. V. Metody biodiagnostiki nazemnykh ekosistem [Biodiagnostics of land ecosystems]. Rostov-on-Don: Southern Federal University, 2016.
15. Kong W.D., Zhu Y.G., Fu B.J., Marschner P. and He J.Z. The veterinary antibiotic oxytetracycline and Cu influence functional diversity of the soil microbial community. Environ. Pollut., 2006, 143: 129–137.
16. Wunder D.B., Tan D.T., LaPara T.M. and Hozalski R.M. The effects of antibiotic cocktails at environmentally relevant concentrations on the community composition and acetate biodegradation kinetics of bacterial biofilms. Chemosphere, 2013, 90: 2261–2266.
17. Denisova T.V., Kolesnikov S.I., Mazanko M.S., Minnikova T.V. and Akimenko Yu.V. Influence of electromagnetic fields of different frequencies on microbiological properties and enzyme activity of ordinary chernozem combined with chemical pollution. 15th International Multidisciplinary Scientific GeoConference SGEM 2015, SGEM2015 Conference Proceedings, June 18-24, 2015, 3(2):183-190.