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Resistance of Microorganisms in Ordinary Black Soil to Contamination with Oxytetracycline and Tylosin (in the Conditions of a Field Model Experiment)

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Abstract

The article is devoted to the problem of soil contamination with antibiotics. In the conditions of a field model experiment, resistance of microorganisms in ordinary black soil to contamination with antibiotics (oxytetracycline, tylosin) in the concentration of 500 mg per 1 kg of soil was studied. By the degree of resistance to antibiotics, the studied eco-trophic groups of microorganisms formed the following sequence: micromycetes > *Azotobacter* bacteria > amylolytic bacteria > ammonifier bacteria. By the speed of recovery, microorganisms formed the following sequence: *Azotobacter* bacteria > micromycetes > amylolytic bacteria > ammonifier bacteria.

Keywords: contamination, microorganisms, ordinary black soil, oxytetracycline, tylosin.

INTRODUCTION

Currently, the problem of environment pollution with veterinary antibiotics and pathogenic microorganisms acquiring resistance to them is paid special attention to. Oxytetracycline and tylosin are widely used worldwide, both for therapeutic purposes, and as growth stimulants. According to the data of *Research Techart* company, the livestock breeding sector in Russia annually uses about 3.5 thous. t of antibiotics. Of these, 23% are used for treatment of diseases and preventive care, 19% are used as growth stimulants, 36% are used as antiparasitic preparations, and 22% are used as prophylactic agents [1]. Since many antibiotics are water-soluble, up to 90% of one dose may be excreted with urine, and 75% - with faeces of animals [2, 3].

The antibiotics mainly get into the soil through the use of manure [4] and wastewater [5] as fertilizers on agricultural lands. Currently, antibiotics are more and more frequently detected in ground and in drinking water, wastewater, and in agricultural soil [6, 7] in the concentrations of up to 300 ng/g^{-1} for tetracycline and 11 ng/g^{-1} for sulfanilamides [8, 9]. High concentrations of tetracyclines were detected in the soils in Turkey and Spain, up to 0.5 mg/kg^{-1} and 0.2 mg/kg^{-1}, respectively [10, 11]. Concentrations of tetracycline (20 mg/l^{-1}) and chlortetracycline (1 mg/l^{-1}) were detected in pig defecators [12]. In addition, various antibiotics are naturally formed in soils; however, concentrations and types of the antibiotics that enter soil and water are very different from the natural background [13].

Currently, agricultural workers are blamed for ingress of farmland antibiotics into the surrounding water bodies. Municipal water treatment systems are unable to filter out antibiotics, and therefore it is very important to understand how such contamination affects the state of ecosystems.

The data about the effect of antibiotics on the soil microbiocenosis have not been found in Russian literature. It is only in some studies that antibiotics were used for determining the ratio of fungi and bacteria in the biomass of various types of soils, and the effect of pharmaceuticals on the biota in water bodies [14-16]. Many results obtained by foreign authors are associated with laboratory modeling of soil contamination with antibiotics. This is due to the fact that laboratory model experiments have certain advantages, compared to field experiments. Firstly, they provide the possibility of maintaining constant soil humidity and temperature, while in the conditions of the field, the results of experiments are highly dependent on the weather conditions. Secondly, they provide the possibility of ensuring soil mixing to complete homogeneity in all vessels, which allows avoiding discrepancies in soil properties. And finally, they provide a possibility of studying various pollutants separately, in predetermined concentrations. Thus, many questions about the effect of pollutants on the processes in the soil may only be answered through laboratory model experiments.

This research was aimed at assessing resistance of microorganisms in ordinary black soil to contamination with antibiotics (oxytetracycline, tylosin) in field conditions.

MATERIALS AND METHODS

Tylosin is a macrolide antibiotic produced by *Streptomyces fradiae*; it is widely used in livestock and poultry breeding as a therapeutic and prophylactic agent and growth stimulant. In Russia, several oral preparations of tylosin are registered: Tilan (Elanco, USA); Tylanic® water soluble powder (VIC group, Russia); Pharmasin (Balkanfarma, Bulgaria). According to the available data, tylosin-based preparations take the leading position in preventing mycoplasmal infections and chronic respiratory disease in poultry. They are active against most Gr^+ and some Gr^- bacteria.

Oxytetracycline is a derivative of tetracycline; it is produced by the *Streptomyces rimosus* fungus. The preparation is produced in the form of sterile yellow crystalline powder – oxytetracycline hydrochloride; it is odorless, and well soluble in water. It is a pluripotent antibacterial preparation. It is active against most Gr^+ and some Gr^- bacteria.

Field model experiments were performed at the Botanic Garden of the Southern Federal University (Rostov-on-Don). The object of the research was ordinary black carbonated soil of the South-European facie. Thickness of the humus layer in black soil was about 80 cm, the granulometric texture was heavy loamy, reaction of the medium was 7.7, humus content was 4.1%, total nitrogen (by Kjeldahl) — 0.25%; total phosphorus (by Ginsburg et al.) — 0.18%; mobile phosphorus (by Machigin) — 28.8%; and total potassium (by Berzelius) - 2.06%. This type of soil was chosen due to the fact that black soil takes the most part of the soil cover in the South of Russia, and is the main land resource for agricultural production [17].

In the field experiments, experimental plots were arranged according to the generally adopted field experiment procedure [18, 19]. Plots with the area of 1 m^2 were used. The experiment was repeated three times. Soil samples for determining biological parameters were taken on day 5, 30, and 60 of the experiment. The average daily air temperature was 18-20°C at the beginning of the experiment, 22-25°C by day 30, and 27-30°C by day 60. Soil humidity was maintained by water irrigation at about 60% of the normal field capacity.

Antibiotics were introduced into the soil in the form of solutions at the concentration of 500 mg per 1 kg of soil. These concentrations were chosen based on literature data about residual amounts of antibiotics in the environment [20], as well as on the results of previously performed exploratory laboratory research [21]. Plots untreated with antibiotics were used for reference.

The laboratory and analytical studies were performed with the use of the methods generally adopted in soil biology and ecology [22, 23]. Comprehensive study of black soil microbiocenosis included determination of microorganisms counts by the method of deep introduction of appropriate dilutions onto solid nutrition media: ammonifying bacteria - onto meat-and-peptone agar, amylolytic bacteria – onto starch-and-ammonia agar, micromycetes – onto acidified Czapek's medium, *R. Azotobacter* bacteria - onto Ashby medium (by the method of lumps fouling).

RESULTS AND DISCUSSION

The field model experiments' results are shown in Fig. 1, 2.

Reaction of the medium (pH) is an important chemical indicator of soils, on the one hand, due to its effect on the content of humic components, and on the other hand, due to its effect on soil microbial activity [24]. In studying the pH of black soil, a trend to lowering the pH towards the acid side is observed (Fig. 1), especially on day 30 of exposure. Most likely, this trend is observed because stable groups of microorganisms remain, which produce compounds containing H^+. Later, reaction of the medium shifts to the initial values. Earlier, in studying the effect of antibiotics on the biological properties of soils during laboratory simulation (benzylpenicillin, tetracycline), the authors showed the pH changes with the duration of exposure toward the alkaline side [25, 26], i.e. pH change depends on the chemical nature of the antibiotic. Thus, antibiotics disrupt the natural medium of soil microorganisms, which causes changes in their counts, and the structure of the microbiocenosis as a whole.

The introduction of antibiotics into the soil resulted in changes in the number of major soil microorganisms; a downward trend in their numbers was observed during all periods of exposure. The studied groups of microorganisms differed by their resistance to the introduced antibiotic (Fig. 2).

Oxytetracycline has the best inhibitory effect on the number of ammonifying bacteria (20-60% of the reference, $p<0.05$, $n=6$) in all periods of exposure. No statistically veracious differences were found in the number ammonifying bacteria on days 5 and 30 of the experiment. On day 60 of the experiment, the number of bacteria in this group was still not restored.

Similar data about the dynamics of the number of ammonifying bacteria were obtained in model experiments with various types of radiation [27]. In case of contamination with heavy metals [28], oil and oil products [29] in relatively small dosages (up to 1 MPC), and immediately after the moment of contamination (up to 1 month), the number of microorganisms in the soil in some cases increases, after which it decreases, and does not restore even after 360 days. In addition, compared to the effect of sterilization with high temperatures [30] and with pesticides [31], the effect of antibiotics on soil microorganisms is more prolonged, since, for example, after soil sterilization with high temperatures, the number of microorganisms recovered to the reference values as early as on day 60 of the experiment.

Prolonged effect of antibiotics on soil microorganisms indicates their accumulation in the soil. Stone et al. [32] detected active metabolites of tylosin 150 days after the antibiotic had been introduced into the soil. Loke et al. [33] showed that with pH below 7.4, strong ionic bonds were formed between protonated tylosin and anionic components of soil, indicating high tylosin sorption in the soil. Positive correlation was detected between tylosin and oxytetracycline sorption and the content of humus, pH and cation exchange capacity of soil [34]. High tylosin sorption capacity was observed in the soils rich in humus, rather than in sandy soils [35, 36].

Amylolytic bacteria were more resistant to the studied antibiotics than ammonifier bacteria. On day 5 of the research, the maximum reduction in the bacteria count was observed (60% of the reference, $p<0.001$, $n=6$). Same as with ammonifier bacteria, the greatest inhibitory effect was observed for oxytetracycline. Further exposure revealed no significant changes in the number of amylolytic bacteria; the trend to restoring the number to the reference values was observed. Other studies showed that antibiotics, such as sulfonamide, tetracycline, trimethoprim, also had inhibiting effect on the number and activity of amylolytic bacteria [37].

As expected, the results of the field modeling of black soil contamination with antibiotics revealed no significant effect of antibiotics on soil micromycetes. The used antibiotics inhibited bacteria growth and development. On day 5 of the experiment, an increased number of micromycetes was observed, compared to the reference. This can be explained by removing the competition with bacteria, and active colonization of the ecological niche with micromycetes. On day 30 and 60 of the experiment, no changes in the number of micromycetes were observed.

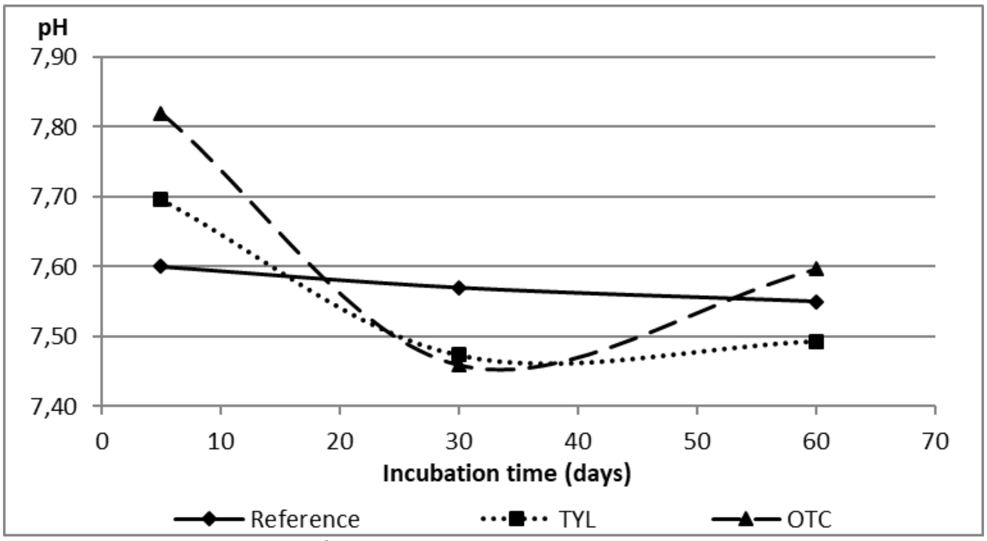

Fig. 1. Changes in the medium reaction (pH) of black soil contaminated with antibiotics (TYL is tylosin, OTC is oxytetracycline)

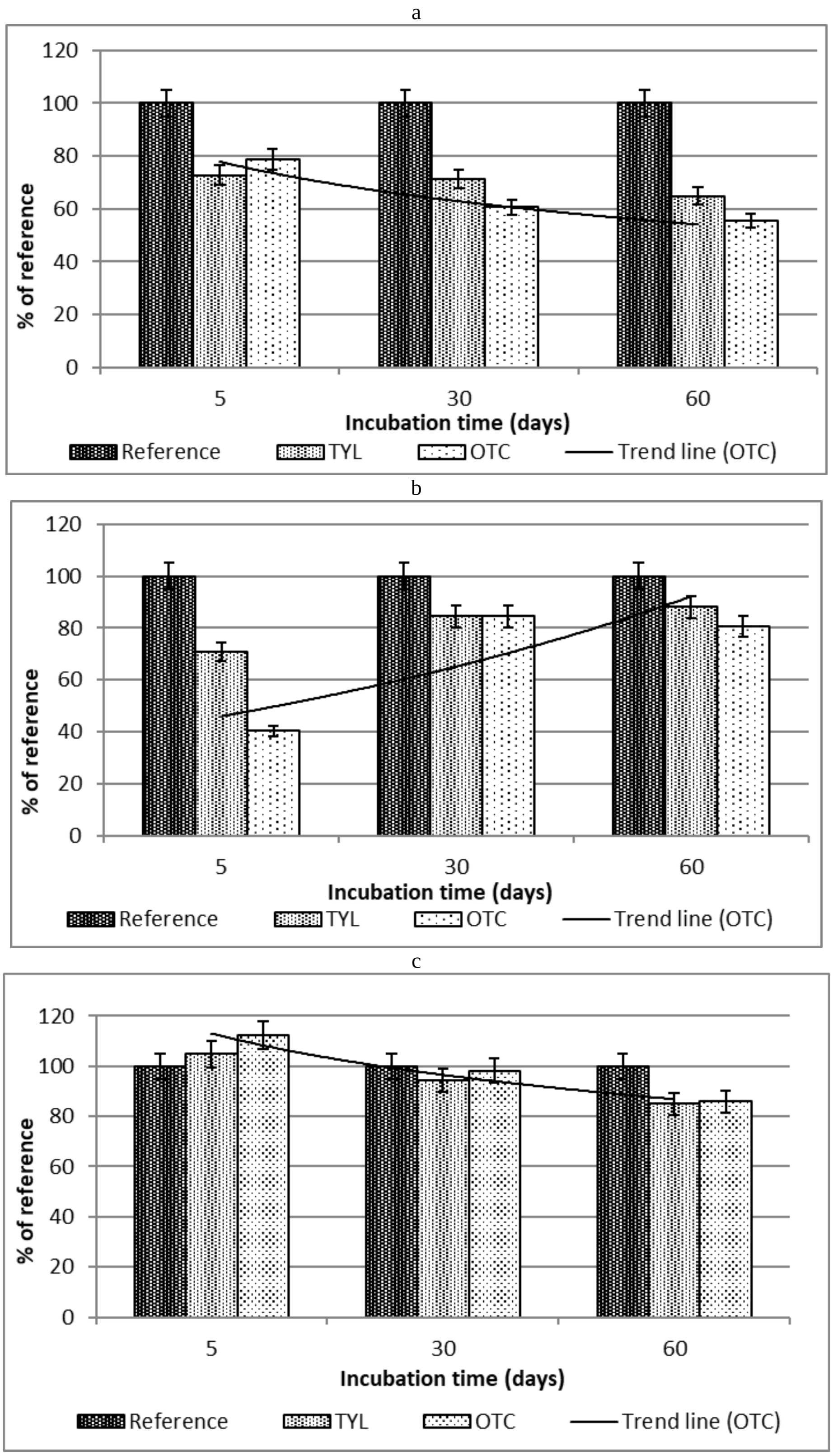

Fig. 2. Changes in the number of microorganisms in black soil upon contamination with antibiotics (a – ammonifying bacteria, b – amylolytic bacteria, c – micromycetes) (TYL is tylosin, OTC is oxytetracycline)

Azotobacter bacteria were less sensitive to the antibiotics introduced. Changes in the number of bacteria were observed only on day 3 of exposure (decrease by 30-40% from the reference when exposed to tylosin, and by 50-60% when exposed to oxytetracycline, respectively); no changes were observed at later dates. With that, nitrogen-fixing bacteria were more sensitive to contamination with heavy metals, oil, ionizing radiation than other groups of bacteria [38, 39].

Thus, the introduction of antibiotics causes changes both in the number of major groups of soil microorganisms and in the structure of microbial coenosis as a whole. In addition, the observed recovery of the population of certain groups of microorganisms is an evidence of the fact that microorganisms acquire resistance to antibiotics. In the research by Y. A. Markova, et al., [40] the resistance of the *Enterobacteriaceae* bacteria to 11 antibiotics isolated from the soil, from tissues, and from the surface of certain cultivated and wild plants was studied.

CONCLUSIONS

By the degree of resistance to antibiotics, the studied microorganisms formed the following sequence: micromycetes > *Azotobacter* bacteria > amylolytic bacteria > ammonifier bacteria. Recovery of the microbiological parameters is not direct, i.e. one cannot say that greater recovery rate occurs with increasing the time of exposure. On the rate of recovery, microorganisms in ordinary black soil have formed the following sequence: *Azotobacter* bacteria > micromycetes > amylolytic bacteria > ammonifier bacteria.

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