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Assessment of Changes in the Biological Indicators of Brown Forest Soil When Polluted with Modern Biocides

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Abstract*: The article is devoted to the actual problem of soil pollution with modern biocides (antibiotics, pesticides). In model laboratory studies, the effect of antibiotic (tylosin) and pesticide (bastion) at concentrations of 1, 10, 100, 1,000 mg/kg on the biochemical properties and microbiocenosis of brown forest soil has been studied. The studies have found negative impact of brown forest soil pollution with biocides resulting in the change of the main microbial and biochemical parameters, indicating the violation of the ecological functions of the soil. The impact of biocides depends on their nature, concentration, and time of exposure. The tylosin antibiotic has stronger inhibitory effect compared with the bastion fungicide. The introduction of low doses of pollutants (1, 10 mg/kg) leads to a slight increase in the abundance of soil microorganisms. Pollution with high doses (100, 1,000 mg/kg) leads to a significant reduction of the abundance of soil microorganisms and enzymatic activity. If polluted with biocides, the environmental conditions (pH) of brown forest soil also change. Enzymes belonging to oxidoreductases class are less resistant to contamination with biocides than hydrolases. The maximum impact of biocides is manifested in the first period after soil pollution (3 days), at subsequent periods there is a tendency to the restoration of biological indicators; however, full recovery (to control values) isn't observed even 90 days after the pollution.

***Index Terms*: pollution, antibiotics, brown forest soil, tylosin, bastion, soil microorganisms, enzymatic activity, luminescence microscopy.**

I. INTRODUCTION

Soil plays an important role in the maintenance of human activity. This very thin layer has the amazing property, not inherent in any other components of our planet – biological productivity. By its nature, the soil is a heterogeneous system that serves as habitat for an enormous number of living organisms, including those producing antibiotics [1 – 4]. With the high ability to adaptation and survival, microbial community of the soil, however, is very vulnerable in the context of chemical pollution. Because of the rapidity of metabolic processes, microbiocenosis is the first to respond to pollution by various toxic substances [5].

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Large-scale development of industry and agriculture entails an increased risk of soil pollution with various chemicals. Near the cities, factories and industrial areas, soils are subjected to a strong anthropogenic load. In the area of enhanced agricultural and livestock activities, a huge amount of biocides (pesticides and antibiotics) enters the soil along with air masses, wastewater and natural fertilizers [6]. The extensive use of antibiotics as growth promoters as well as to combat various diseases in livestock farming entails the growing problem of antibiotic resistance and the threat to human health worldwide. Pesticides have been used in agriculture for many years, and their negative impact on the flora and fauna has long been established. Particularly toxic pesticides, such as DDT, endrin, mirex, toxaphene, are prohibited for use in many countries around the world.

Antibiotics have been found in soils everywhere in high concentrations, even far from agricultural complexes [7, 8]. On a scale of vast cattle farms, it is difficult to quantitatively control the use of antibiotics and pesticides; thus, together with sewage and manure, they fall into natural ecosystems and can quickly accumulate on different trophic levels. In many European countries, the prohibition to use antibiotics as growth promoters and feed additives is enshrined in law. The Russian Federation lacks such regulations and every year the number of antibiotics that are defined in different ecosystems increases [9, 10]. Thus, the study of the effects of biocides (antibiotics and pesticides) on biological indicators of soil seemed to be relevant in the context of the rapidly developing agriculture, as well as due to the growing problem of antibiotic resistance. The purpose of this study is to assess changes in biological indices of brown forest soil at pollution with modern biocides (tylosin, bastion) at concentrations of 1, 10, 100, 1,000 mg/kg of soil over time (3, 30 and 90 days).

II. MATERIALS AND METHODS

The bactericidal veterinary antibiotic (tylosin) and the systemic fungicide (bastion), widely used in veterinary medicine and agriculture, were chosen as biocides. Tylosin is a macrolide antibiotic produced by Streptomyces fradiae, widely used in animal husbandry as a therapeutic and prophylactic agent and growth stimulant. It is active against most Gram-positive and some Gram-negative bacteria. Bastion is a combined systemic-action fungicide to combat pathogens of fungal diseases spreading with seeds and through the soil.

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It is effective against ascomycetes, basidiomycetes and imperfect fungi.

The object of the study was brown forest soil that had been selected in the Republic of Adygea in the Nickel settlement (0 – 20 cm). This soil is very fertile, subject to intensive cultivation, and is widespread in the Caucasus regions, as well as in the Far East. In the North Caucasus, this type of soil is widely used by forestry fund and nature reserves. The natural vegetation of these soils in most regions is represented by broad-leaved beech-oak, beech-hornbeam, and beech-ash forests [11]. Air-dry soil samples were polluted with a solution of the tylosin antibiotic and the bastion fungicide (at the concentrations of 1, 10, 100, 100 mg/kg of soil). Studies were conducted at 3, 30 and 90 days after the introduction of

pollutants. Soil samples not polluted with biocides served as controls. Concentrations and terms of incubation were selected based on the previous reconnaissance studies, as well as on literature data [12 – 15].

To assess the effect of biocides, the following biological indicators were selected: microbiological – the total number of bacteria, the abundance of Azotobacter bacteria; biochemical – the activity of oxidoreductase enzymes (catalase, dehydrogenase), hydrolase enzymes (invertase, phosphatase), and the pH value. The total number of bacteria was studied by luminescence microscopy with staining with fluorochrome dyes with acridine orange. The luminous green cells of bacteria were counted on the ZEISS AXIO Vert A1 inverted microscope with a 450 – 490 nm light filter. When counting cells, 20 visual fields for each slide were viewed. The number of cells per 1 g of soil was calculated according to the following formula:

$N = S1*a *n/v*S2*c$,

where N was the cell number per 1 g of soil; S1 was the agent area (μm2); a was the number of cells in one field of view (averaging is performed for all drugs); n was the dilution rate of soil suspension (ml); v was the volume of the drop applied to the glass (ml); S2 was the microscope field of view (μm2); and c was the soil lot (g).

The abundance of Azotobacter bacteria was studied by the fouling method on the Ashby nutrient medium. Catalase and dehydrogenase activity was studied by the Galstyan method as modified by Khaziyev; the invertase activity was studied using the modified Khaziyev colorimetric method, that of phosphatase – by the modified Galstyan and Arutyunyan method; and the soil medium (pH) was studied using potentiometric method [16]. The results were statistically processed using Statistica 10.0 package and Excel. The main indicators of variation statistics were calculated: mean (μ), standard error (μ ± m), standard deviation (s), the coefficient of variation (CV) and others.

III. RESULTS AND DISCUSSION

The greatest number of microorganisms lives in the area of distribution of the root system of plants – the rhizosphere. The analysis of the total number of bacteria using luminescent microscopy has revealed that pollution of brown forest soil at all incubation periods, at concentrations of 1 and 10 mg/kg, does not lead to a significant change in the total number of bacteria, and a slight increase in their numbers is observed compared with the control (5 – 10 %). The obtained data testify to the "low dose effect" and are confirmed by the work with other chemical pollutants [17, 18]. A close inverse correlation has been established between the concentration of biocides and bacterial count (r = -0.80). On the 30th day of the study, a decrease in the number of bacteria was observed in all soil samples, relative to the controls (by 20 – 40%). Ninety days after the incubation, the total number of bacteria was reduced by 10 – 20% of the control. However, there is a general trend of recovery in the total number of microorganisms throughout the incubation period. Of the studied biocides, the tylosin antibiotic has greater inhibitory effect on the bacterial count of brown forest soil than the bastion fungicide. The soil is heavily populated by many bacteria, including p.Azotobacter being the Gram negative bacteria living in the soil. The peculiarity of this type of bacteria is that its representatives transform atmospheric nitrogen from atmospheric nitrogen N2, which is very hardly assimilated by living organisms, into forms of nitrogen compounds that are more accessible for use. Bacteria of this genus are among the few free-living nitrogen-fixing agents. Due to this feature, these bacteria increase the fertility of the soil and are widely used in agricultural activities, as a soil quality indicator [19]. In the framework of the studies in the first incubation period, three days after the introduction of pollutants, a slight increase in the abundance of p.Azotobacter bacteria in the soil samples contaminated with biocides was found at a concentration of 1 mg/kg of soil compared with control samples (Fig. 1, 2). In the samples polluted with biocides at concentrations of 10 and 100 mg/kg of soil, a slight decrease in the abundance of bacteria was observed relative to the control. At a concentration of 1,000 mg/kg of soil, these biocides had a significant inhibitory effect on the abundance of bacteria of this genus, reducing it by an average of 70 – 80%. At the incubation period of 30 days, significant fluctuations in the number of bacteria were observed when polluted with biocides at various concentrations. In the soil samples polluted with biocides at a concentration of 1 mg/kg of soil, a decrease in the count was observed relative to the first incubation period. While in the samples contaminated with biocides at a concentration of 1,000 mg/kg of soil, on the contrary, an increase in the count of bacterial populations was observed. These changes in count can be associated with the phenomenon of population waves and the gradual recovery of the population size, its value approaching the average after a sharp decrease and increase in the count of the *p.Azotobacter* bacteria, which was observed three days after the pollutant had been introduced into the soil. With increasing the time of incubation of the polluted samples, tendencies to restore the abundance of this kind of bacteria were noted. In the laboratory studies on the 90th day, there was an approximately equal number of bacteria in all samples, regardless of the initial concentration of the pollutant. There was a slight increase in the abundance of the *p.Azotobacter* bacteria compared with the control samples. Apparently, this may be due to the high rate of adaptation of microorganisms to pollution with biocides, as well as the gradual decomposition of the pollutants introduced into the soil.

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The resistance of the *p.Azotobacter* bacteria to antibiotics in low concentrations was also observed in the studies of other authors [18].

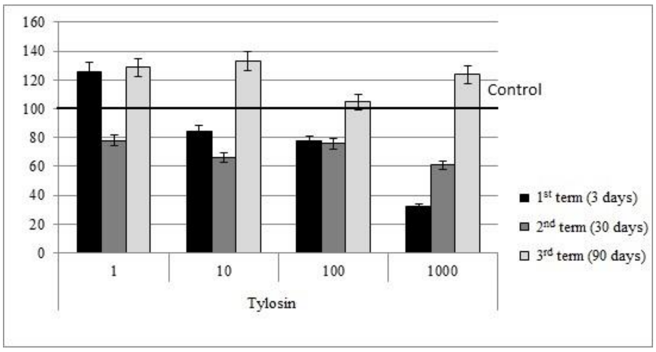

Fig. 1. Dynamics of the abundance of *p.Azotobacter* bacteria in brown forest soil when polluted with tylosin, % of control

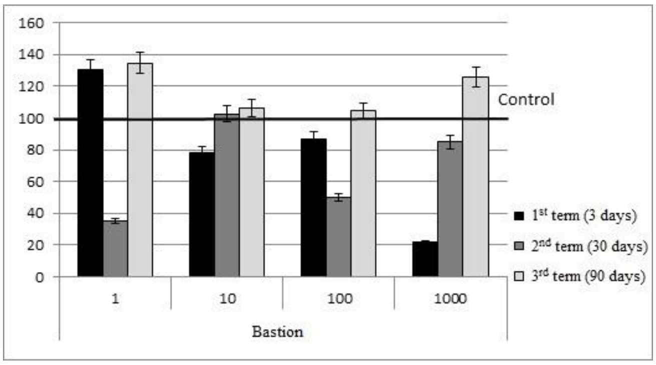

Fig. 2. Dynamics of the abundance of *p.Azotobacter* bacteria in brown forest soil when polluted with bastion, % of control

On the third day after the introduction of the pollutants at a concentration of 1, 10 mg/kg of soil, an increase in the activity of catalase and dehydrogenases was noted (10 – 20%). In the scientific literature, an increase in the values of indicators when introducing insignificant concentrations of a pollutant is called the "low dose effect". Moreover, this effect was more pronounced in the samples of soil polluted with bastion fungicide: an increase of 40 – 45% was observed, while with the introduction of the tylosin antibiotic it was 10 – 20%. Pollution of brown forest soil with high doses of biocides of 100 and 1,000 mg/kg leads to a significant decrease in the enzymes of this class (20 – 30% for catalase, 40 – 50% for dehydrogenases). On the 90th day, there is a recovery in the oxidoreductases activity.

When studying the invertase activity in the first incubation period, the activity of this enzyme in all samples decreased as compared to the control. An inverse correlation of the enzyme activity with the concentration of the pollutant was revealed. The smallest decrease in activity was observed when introducing biocide at a concentration of 1 mg/kg soil, and the highest one – at a concentration of 1,000 mg/kg soil. At the subsequent incubation period of 30 and 90 days after the biocide introduction, the recovery of the enzyme activity was observed. On the third day after the pollution with biocides at concentrations of 1 and 10 mg/kg, there is a slight increase in the phosphatase activity compared with the control (by 5 – 10%), high doses of biocides reduce the activity of the enzyme by 10 – 30%, with the greatest reduction in the enzyme observed in tylosin-polluted samples.

The hydrogen ions in the soil solution, as well as the exchangeable ions of hydrogen and aluminum in the absorbing complex, with incomplete neutralization, form an acid reaction of the medium in the soil. Increased acidity of the soil affects the growth and development of most cultivated plants [20]. Analysis of the results showed that the change in the medium reaction (pH) of brown forest soil depended on the chemical nature and the concentration of biocides introduced. Pollution with tylosin antibiotic leads to a slight shift of pH to the alkaline side, while the bastion fungicide at low concentrations does not significantly affect the acidity, but at high concentrations leads to a shift of pH to the acidic side. The significant change in the reaction of the medium (pH) of brown forest soil occurs at pollution with biocides at a concentration of 100 mg/kg. Thus, pollution with biocides leads to a change in the acid-base balance, followed by the disruption of the natural medium of soil microorganisms, which results in the change of the activity of the soil microbiota, which performs diverse functions in the circulation of substances, including self-purification from various xenobiotics and pollutants. Similar results were obtained in other studies [21 – 23].

IV. CONCLUSION

The change in microbiological and biochemical parameters of brown forest soil at pollution with modern biocides has been determined. The impact of biocides depends on their nature, concentration and time of exposure. The tylosin macrolide antibiotic has stronger effect compared with the bastion fungicide. The introduction of low doses of biocides (1, 10 mg/kg of soil) leads to a slight increase in the number of soil microorganisms, the count of p.Azotobacter bacteria and enzymatic activity. In the scientific literature, this phenomenon is called the "low dose effect". Enzymes from the oxidoreductases class (catalase, dehydrogenases) have been less sensitive to contamination than hydrolases (invertase, phosphatase). Pollution with tylosin leads to a slight shift of pH to the alkaline side, and that with bastion (in high concentrations) leads to the pH shift to the acid side. The significant change in the reaction of the medium (pH) of brown forest soil occurs at pollution with biocides at a concentration of 100 mg/kg.

The maximum impact of biocides is manifested in the first periods after the pollution of soil (3 days), and in the subsequent periods (30, 90 days) there is a tendency to restore the studied parameters. However, full recovery (to control values) has not been observed even 90 days after the pollution.

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