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Research Article

Silver Ecotoxicity Estimation by the Soil State Biological Indicators

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The use of silver in various spheres of life and production leads to an increase in environmental pollution, including soil. At the same time, the environmental consequences of silver pollution of soils have been studied to a much lesser extent than those of other heavy metals. The aim of this study is to estimate silver ecotoxicity using the soil state biological indicators. We studied soils that are significantly different in resistance to heavy metal pollution: ordinary chernozem (Haplic Chernozems, Loamic), sierosands (Haplic Arenosols, Eutric), and brown forest acidic soil (Haplic Cambisols, Eutric). Contamination was simulated in the laboratory. Silver was introduced into the soil in the form of nitrate in doses of 1, 10, and 100 mg/kg. Changes in biological parameters were assessed 10, 30, and 90 days after contamination. Silver pollution of soils in most cases leads to deterioration of their biological properties: the total number of bacteria, the abundance of bacteria of the genus *Azotobacter*, the activity of enzymes (catalase and dehydrogenases), and the phytotoxicity indicators decrease. The degree of reduction in biological properties depends on the silver concentration in the soil and the period from the contamination moment. In most cases, there is a direct relationship between the silver concentration and the degree of deterioration of the studied soil properties. The silver toxic effect was most pronounced on the 30th day after contamination. In terms of their resistance to silver pollution, the studied soils are in the following order: ordinary chernozem > sierosands ≥ brown forest soil. The light granulometric composition of sierosands and the acidic reaction of the environment of brown forest soils, as well as the low content of organic matter, contribute to high mobility and, consequently, high ecotoxicity of silver in these soils. The regional maximum permissible concentration (rMPC) of silver in ordinary chernozem (Haplic Chernozems, Loamic) is 4.4 mg/kg, in sierosands (Haplic Arenosols, Eutric) 0.9 mg/kg, and in brown forest soils (Haplic Cambisols, Eutric) 0.8 mg/kg.

1. Introduction

The silver technophility over the past 50 years has been growing at an exponential rate and, according to forecasts, will only increase soon [1]. The main anthropogenic sources of silver pollution of the environment, including soils, are emissions from thermal power plants during coal combustion [2–6], nonferrous and ferrous metallurgy enterprises [7], cement plants [8], solid waste landfills [9], production of photo and electrical materials [10], pesticides [11], the use of sewage sludge as fertilizers [12], etc. The extent and degree of silver pollution in soils are increasing every year [13, 14].

In modern conditions of nanotechnology development, silver nanoparticles are increasing sources of environmental pollution [15–18].

Silver toxicity has been established for bacteria [19–23], plants [16, 24–26], nematodes [27], earthworms [28], mollusks [29], fish [30, 31], rats [32, 33], mice [34], and human [35, 36]. Silver ions Ag^{+} possess genotoxic properties [37, 38].

Silver is capable of interacting with various proteins [39–41], and, therefore, the mechanism of silver toxicity is apparently the same as that of other heavy metals and metalloids—inhibition of enzymes and a decrease in the permeability of biological membranes [12, 32, 33], DNA damage [30, 42], metabolic disturbance [29, 42], and cell necrosis [33].

At the same time, the environmental effects of silver soil pollution have been studied to a much lesser extent than those of other heavy metals such as lead, cadmium, and mercury. Therefore, it seems relevant to identify patterns of the effect of silver on the state of soils depending on the dose of metal and the period from the moment of contamination, to establish limits on the resistance of different soils to pollution, and to normalize the silver content in soils.

The aim of this work is to assess the ecotoxicity of silver by biological indicators of soil condition, which differ significantly in the degree of resistance to pollution.

2. Materials and Methods

Soils of the south of Russia, significantly differing in properties determining the resistance to heavy metal pollution, were used as objects of study: ordinary chernozem (Botanical Garden of SFU, the Rostov-on-Don city, 47°14′17.54″ north latitude, 39°38′33.22″ east longitude), sierosands (the Rostov Region, the Ust-Donetsk Region, Verkhnekundryuchenskaya station, 47° 46.015′ north latitude, 40° 51.700′ east longitude), and brown forest acidic soil (the Republic of Adygea, the Maykopsky district, Nikel settlement, 44° 10.649′ north latitude, 40° 9.469′ east longitude).

Ordinary chernozem (according to IUSS Working Group WRB [43]—Haplic Chernozems, Loamic) is characterized by a heavy loam granulometric composition, an average organic matter content of 3.7%, and a neutral reaction of the medium pH = 7.8. Sierosands (Haplic Arenosols, Eutric) are characterized by light particle size distribution; low organic matter content, 2.3%; and neutral pH, 6.8. Brown forest soils (Haplic Cambisols, Eutric) are characterized by heavy loam granulometric composition; low humus content, 1.8%; and acid reaction of the medium, 5.8.

Silver soil contamination was modeled in the laboratory. The soil was taken from the top layer 0–10 cm because silver usually accumulates in the surface layers of the soil [44].

According to different authors, the silver content in unpolluted soil is 0.01–1 mg/kg [45, 46], from 0.07 to 0.1 mg/kg [44]. According to various sources, the content of silver in contaminated soils is up to 8 mg/kg [47], 9 mg/kg [45], 19.5 mg/kg [48], 23 mg/kg [49], and 35.9 mg/kg [50], and it is up to 7000 mg/kg in the soils of ore deposits [51].

Since the maximum permissible concentration (MPC) of silver in the soil has not been developed, its content in the soil was expressed in the conditionally permissible concentration (CPC). CPC values were taken equal to three background silver concentrations in the soil. This is because the MPCs of most heavy metals and metalloids are about three to four background concentrations in the soil [52]. The background silver content in ordinary chernozem is 0.303 mg/kg, brown forest soil 0.282 mg/kg, and sulphurous sand 0.215 mg/kg (silver content in soils was determined by inductively coupled plasma mass spectrometry). Accordingly, CPC was taken equal to 1 mg/kg. Silver was introduced into the soil in the amount of 3.30 and 300 background concentrations (1.10 and 100 mg/kg, respectively). Since the silver content in contaminated soils often reaches 35 mg/kg [50] and in the soils of ore deposits up to 7000 mg/kg [51], the studied concentrations and even high silver concentrations in the soil are already found. In addition, one of the objectives of the study is to forecast the possible negative consequences of this level of pollution.

Silver was introduced into the soil in the form of nitrate ($AgNO_3$). When contaminated, silver enters the soil in the form of sulfates and sulfides [53] and more recently in the form of nanoparticles [17, 18]. Many authors consider silver nitrate to be the most toxic compound [24, 54, 55]. Silver nitrate is a highly soluble substance in water. This allows you to evaluate the maximum toxicity of silver, as well as to achieve a uniform distribution of silver in the soil.

Each soil sample (weighing 1 kg) was incubated in plastic vessels in triplicate at room temperature (20–22°C) and optimal moisture (60% of field moisture capacity).

The biological properties of the soil were investigated first since they were the first to respond to external influences. They are significantly more sensitive and informative compared to other soil properties [56].

In the first model experiment, the change in the biological parameters of different soils of the south of Russia (ordinary chernozem, brown forest soil, and sulphurous sand) was assessed during short-term exposure (10 days) of the pollutant. In the second model experiment, the change in the biological parameters of ordinary chernozem was evaluated 10, 30, and 90 days after contamination. The objective of the first experiment was to compare the stability of three different soils, which differ significantly in resistance to chemical pollution. The objective of the second experiment was to investigate the dynamics of changes in the state of soil over time.

After this period, the entire mass of soil was removed from the vegetation vessel and mixed, thereby obtaining a "medium sample" from which samples were taken to determine biological indicators—3 samples from each vessel.

Laboratory and analytical studies were performed using generally accepted methods. Silver toxicity was assessed using biological analysis methods. The total number of bacteria, the abundance of bacteria of the genus *Azotobacter*, the activity of catalase and dehydrogenases, and the phytotoxic properties of the soil (root length) were determined. The total number of bacteria in the soil was determined by luminescence microscopy ($n = 720$: 3 vegetation vessels with soil × 3 soil samples × 4 square centimeters on glass slides × 20 visual fields); the abundance of bacteria of the genus *Azotobacter* was determined by the method of fouling lumps on Ashby medium ($n = 225$: 3 vegetation vessels with soil × 3 soil samples in Petri dishes × 25 lumps of fouling); catalase activity was determined according to the rate of decomposition of hydrogen peroxide ($n = 36$: 3 vegetation vessels with soil in 3 biological replicates × 4 analytical replicates); actively dehydrogenases was determined according to the rate of conversion of triphenyl tetrazolium chloride to triphenyl formazan ($n = 36$: 3 vegetation vessels with soil in 3 biological replicates × 4 analytical replicates);

Table 1: Change in environmental indicators of soils in the south of Russia with silver pollution over 10 days of the experiment.

Soil type	The silver content in the soil, mg/kg				
	Control	1	10	100	$LSD_{0.05}$
The total number of bacteria, billion in 1 g of soil					
Ordinary chernozem	5.1	4.5	3.9	2.6	0.3
Sierosands	2.8	2.1	1.7	1.4	0.2
Brown forest soil	1.9	1.5	1.0	0.9	0.1
$LSD_{0.05}$		0.3	0.2	0.2	
Catalase activity, ml O_2 per 1 g of soil for 1 min					
Ordinary chernozem	11.3	10.7	10.4	10.1	0.9
Sierosands	2.9	2.5	2.3	2.1	0.2
Brown forest soil	2.9	2.4	2.3	2.1	0.2
$LSD_{0.05}$		0.4	0.4	0.4	
The activity of dehydrogenases, mg TFF per 10 g of soil in 24 hours					
Ordinary chernozem	26.1	25.1	23.6	11.6	2.2
Sierosands	21.5	18.1	13.2	12.8	1.7
Brown forest soil	20.7	16.3	12.0	8.6	1.5
$LSD_{0.05}$		2.1	1.7	1.2	
Length of the radish roots, %					
Ordinary chernozem	100	95	92	83	11
Sierosands	100	99	88	76	12
Brown forest soil	100	95	76	71	10
$LSD_{0.05}$		10	9	8	
Integral indicator of the biological state of soils, %					
Ordinary chernozem	100	97	90	73	11
Sierosands	100	87	73	61	10
Brown forest soil	100	85	70	58	9
$LSD_{0.05}$		11	9	8	

and soil phytotoxicity was judged by the germination of radish seeds (*Raphanus sativus* L.) variety (16 days) and root length ($n = 225$: 3 vegetation vessels in 3 biological replicates in Petri dishes × 25 radish seeds).

The choice of biological indicators is due to the following reasons. The total number of bacteria in the soil characterizes the state of reducers in the ecosystem. Bacteria of the genus *Azotobacter* are traditionally used as an indicator of chemical pollution of the soil. The activity of catalase and dehydrogenases reflects the intensity of mineralization processes in the soil. Oxidoreductases are the most sensitive to chemical pollution among enzymes [56]. Enzyme activity is an indicator of the potential biological activity of the soil. The length of radish roots was characterized by a more sensitive indicator than the length of shoots for the presence of toxicants in the soil [57]. The presented set of indicators gives an informative picture of the biological processes taking place in the soil and its ecological state.

Based on the above biological indicators, the integral indicator of the biological state (IIBS) of the soil was determined [56]. For the calculation of IIBS, the value of each of the above indicators on the control (in unpolluted soil) was taken as 100% and, relative to it, the values of other experimental variants (in polluted soil) were expressed as a percentage. Then, the average value of five selected indicators for each experiment was determined. The obtained value (IIBS) is expressed as a percentage concerning the control (to 100%). The methodology used allows the integration of the relative values of different indicators, the absolute values of which cannot be integrated since they have different units of measurement.

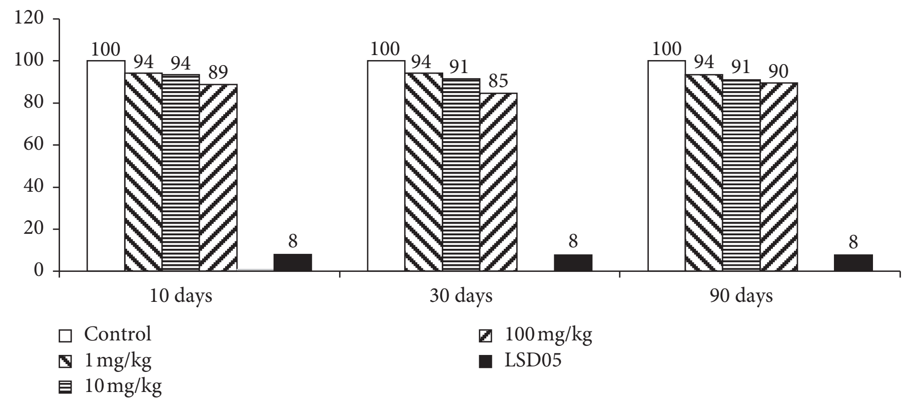
To verify the obtained data for reliability, analysis of variance was carried out with the subsequent determination of the smallest significant difference (LSD).

3. Results and Discussion

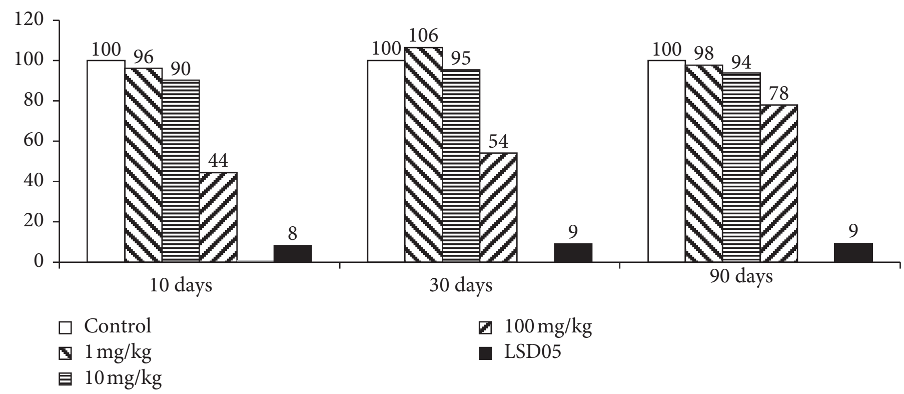
It was found that silver pollution in most cases leads to deterioration in the biological properties of soils in the south of Russia (Table 1, Figure 1). The degree of reduction in biological properties depends on the silver concentration in the soil and the period from the contamination moment. In most cases, there is a direct relationship between the silver concentration and the degree of deterioration of the studied soil properties.

The total number of bacteria, the abundance of bacteria of the genus *Azotobacter*, the activity of catalase and dehydrogenase, and the length of radish roots are reduced.

Only in one variant of the experiment with a dose of 1 CPC of silver on the 30th day after contamination and only on ordinary chernozem, there was a statistically unreliable stimulating effect of silver on the activity of dehydrogenases recorded. Moreover, there are cases when small doses of silver stimulated the activity of urease and phosphatase [58]; the length of the roots of radishes, wheat, beans, and corn [59, 60]; and nitrification process [61]. However, in the present study, stimulation only on the dehydrogenase activity was recorded.



(a)



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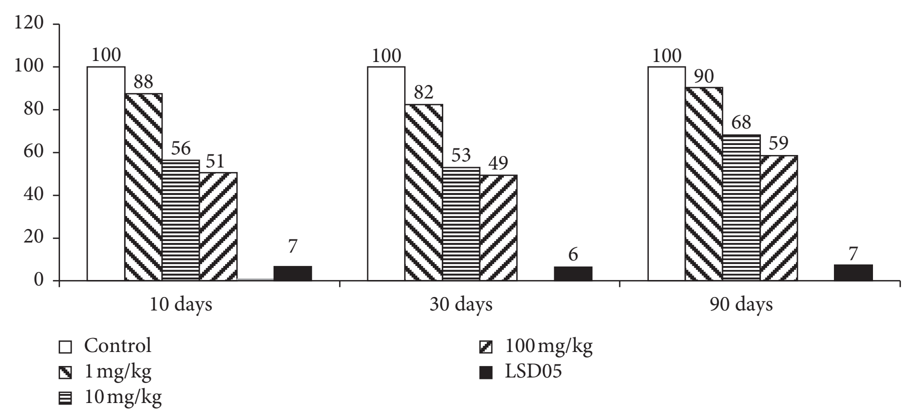

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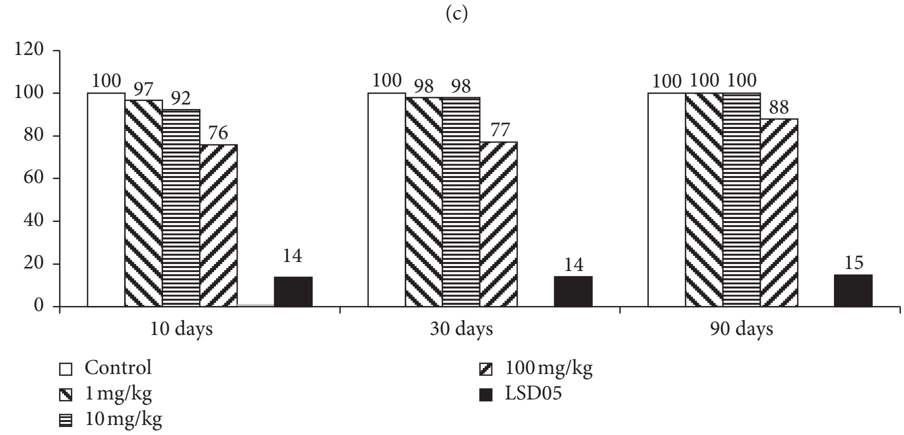
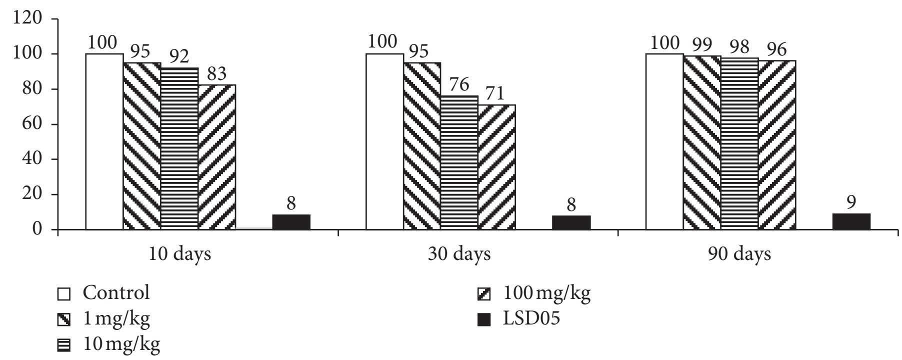


(d)

Figure 1: Continued.



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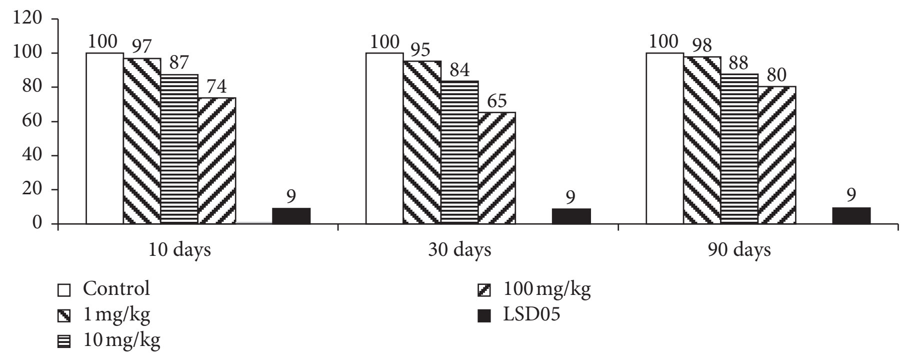

(f)

Figure 1: Changes in the biological state of chernozem 10, 30, and 90 days after silver contamination. (a) Catalase activity, (b) activity of dehydrogenases, (c) total number of bacteria, (d) the abundance of bacteria of the genus *Azotobacter*, (e) length of the radish roots, %, and (f) integral indicator of the biological state of soils (IIBS).

When comparing the resistance of the three types of soils to silver pollution, the following order was obtained: ordinary chernozem > sierosands ≥ brown forest soil. Light granulometric composition of sierosands and the acidic reaction of brown forest soils (pH = 5.8), as well as low content of organic matter (1.8% and 2.3%, respectively), contribute to high mobility and, consequently, high ecotoxicity of silver in these soils.

When assessing the dynamics of the biological state of the ordinary chernozem, it was noted that, for most biological indicators, the strongest decrease was recorded on the 30th day after pollution. On the 90th day, there was a tendency to restore the biological properties of ordinary chernozem, but the control values (before soil contamination) were not achieved. Similar regularities in the dynamics of biological properties of soils after pollution were obtained earlier for other heavy metals: Hg, Cd, Pb, Cr, Cu, Zn, etc. [62–64].

The biological indicators of soil contamination with silver were evaluated for their information content and sensitivity to determine the effectiveness of their use in monitoring, diagnosis, and regulation of soil contamination with silver.

The sensitivity of the indicator was assessed by the degree of decrease in its values in the variants with pollution compared to the control (Table 2).

With regard to the degree of sensitivity to soil pollution with silver, the biological indicators of ordinary chernozem can be ordered as follows:

> the total number of bacteria (66) > dehydrogenase activity (84) > radish root length (89) > catalase activity (91) >, abundance of bacteria of the genus *Azotobacter* (92)

The information content of the indicator was evaluated based on the tightness of the correlation between the indicator and the silver content in the soil (Table 3).

With respect to the degree of informative value, the biological indicators of ordinary chernozem form the following sequence:

> dehydrogenase activity (−0.99) ≥ abundance of bacteria genus *Azotobacter* (−0.99) > radish root length (−0.86) > catalase activity (−0.76) >, the total number of bacteria (−0.72)

Previous studies [66] have shown that soil pollution disrupts its ecosystem (biogeocenotic) functions. Violation of the ecosystem functions of the soil depends on the concentration of a pollutant in the soil. Violation of the ecosystem functions of the soil has the following sequence. First, there is a breakdown of information functions; then biochemical, physicochemical, chemical, and integral functions; and then physical functions. In environmental regulation of soil pollution, depending on the dose of pollutant in the soil, it is advisable to use the established pattern of disruption of ecosystem functions of the soil. Soil IIBS is used as an indicator of disturbance in the ecosystem functions of the soil. With a decrease in IIBS by less than 5%,

Table 2: Assessment of biological indicators of soil condition during silver contamination in terms of sensitivity and informative value.

Indicator	Sensitivity[1]	Informative value[2]
Number of bacteria	66	−0.72
The abundance of bacteria of the genus *Azotobacter*	92	−0.99
Catalase activity	91	−0.76
Activity of dehydrogenases	84	−0.99
Length of the radish roots	89	−0.86
IIBS	85	−0.91

[1]The sensitivity of the indicator is the degree of decrease in the biological indicator when the soil is contaminated with silver, % of the control (values are averaged over the doses and terms of pollution). [2]The indicator informative value is the correlation coefficient (r) between the content of silver in the soil and the biological indicator (=0.05).

Table 3: The scheme of environmental regulation of silver pollution of soils of Southern Russia by the degree of violation of ecosystem (biogeocenotic) functions.

Soils	Not polluted	Slightly polluted	Medium polluted	Highly polluted
Degree of soil IIBS reduction[1]	<5%	5–10%	10–25%	>25%
Violated ecosystem functions[2]	—	Information	Chemical, physicochemical, biochemical; holistic	Physics
Soils			The silver content in the soil, mg/kg	
Ordinary chernozem	<0.5	1.5–4.4	4.4–106	>106
Sierosands	<0.3	0.5–0.9	0.9–8	>8
Brown forest soil	<0.3	0.4–0.8	0.8–6	>6
Soil remediation methods	Not required	Phytoremediation, flushing	Chemical reclamation: the application of organic substances, ion exchange resins, phosphate fertilizers, lime, zeolites, etc.	Removing contaminated soil and replacing it with a new environmentally and agriculturally sound soil

[1]Determination of IIBS of soils according to Kolesnikov et al. [52]. [2]Classification of ecosystem functions of soil by Dobrovolsky and Nikitin [65].

a disturbance in the ecosystem functions of the soil does not occur, but a decrease in the value of IIBS by 5–10% reveals a violation of information functions; by 10–25% biochemical, physicochemical, chemical, and integral functions; and more than 25% physical functions [52].

An important task of environmental regulation should be monitoring the basic ecosystem functions of the soil and preventing their violation. Thus, a decrease in IIBS by more than 10% indicates serious disturbances in the functioning of the soil. The dose of a soil polluting substance, causing a 10% decrease in soil IIBS, can be considered the rMPC of this substance in this soil, the excess of which is unacceptable.

To calculate the concentration of a pollutant, which causes a different degree of reduction in the soil IIBS, one can apply regression equations, which describes the dependence of the decrease in the values of IIBS on the content of a pollutant in the soil. Using the regression equation, you can calculate the concentration of pollutant that causes a violation of certain groups of ecosystem functions of the soil.

As can be seen from Table 2, if, for example, in ordinary chernozem, the silver content does not exceed 0.5 mg/kg, then the ecological functions of the soil are not disrupted. However, if the silver concentration is between 1.5 and 4.4 mg/kg, there will be a violation of the environmental information functions of the soil; 4.4–106 mg/kg, the chemical, physicochemical, biochemical, and integral, along with the information, functions will be violated; and more than 106 mg/kg, the physical functions of the soil will also be disrupted. It is obvious that the violation of chemical, physicochemical, biochemical, and most importantly integral functions of the soil, which ensure soil fertility, cannot be allowed. The silver concentration of 4.4 mg/kg should be considered the maximum permissible concentration (MPC) of silver in ordinary chernozem, or regional MPC (rMPC).

Thus, the rMPC of silver in ordinary chernozems is 4.4 mg/kg of silver in the soil, in sierosands 0.9 mg/kg, and in brown forest soils 0.8 mg/kg.

Established rMPC can be used in many environmental activities, such as environmental impact assessment (EIA), monitoring of soils and ecosystems, choice of methods of reclamation of silver-contaminated soils, risk assessment of man-made disasters, environmental expertise, and certification.

The rMPC developed can be used not only for soils of the South of Russia but also for similar soils in other regions of the world.

The most effective methods of soil rehabilitation in the south of Russia in the case of their pollution with silver in a certain concentration are presented in Table 3. The higher the concentration of silver in the soil, the more "radical" the method of rehabilitation should be. For example, if the silver content in ordinary chernozem is less than 0.5 mg/kg and there is no violation of environmental functions, then soil sanitation is not required in this case. If the silver concentration is 1.5–4.4 mg/kg, then phytoremediation and washing are sufficient to reduce its concentration to 0.5 mg/kg or less. If the silver concentration reaches 4.4–106 mg/kg, then the introduction of organic substances, ion exchange resins, phosphorus fertilizers, lime, zeolites, etc. is required. If the silver content exceeds 106 mg/kg, it is necessary to remove the contaminated soil layer and replace it with a new environmentally friendly and agricultural soil.

4. Conclusions

Silver pollution of the south of Russia soils in most cases leads to deterioration of their biological properties: the total number of bacteria, the abundance of bacteria of the genus *Azotobacter*, the activity of enzymes (catalase and dehydrogenases), and the phytotoxicity indicators decrease. The degree of reduction in biological properties depends on the silver concentration in the soil and the period from the contamination moment. In most cases, there is a direct relationship between the silver concentration and the degree of deterioration of the studied soil properties. The silver toxic effect was most pronounced on the 30th day after contamination.

When comparing the resistance of soils in the south of Russia to silver contamination, the following sequence was obtained: ordinary chernozem > sierosands ≥ brown forest soil. The light granulometric composition of sierosands and the acidic reaction of the environment of brown forest soils, as well as the low content of organic matter, contribute to high mobility and, consequently, high ecotoxicity of silver in these soils.

The biological indicators used in this study have a high correlation coefficient with silver soil contamination and high sensitivity to silver soil pollution. It is advisable to use these biological indicators to monitor, diagnose, indicate, and normalize silver soil pollution.

The regional maximum permissible concentration (rMPC) of silver in ordinary chernozem (Haplic Chernozems, Loamic) is 4.4 mg/kg, in sierosands (Haplic Arenosols, Eutric) 0.9 mg/kg, and in brown forest soils (Haplic Cambisols, Eutric) 0.8 mg/kg.

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 authors declare that there are no conflicts of interest regarding the publication of this article.

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