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Effect of bismuth contamination on soil biological properties

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Abstract

Importance of the work: Bismuth (Bi) compounds are now used increasingly in various industries as an alternative to toxic lead.

Objective: To study the effect of bismuth contamination on soil biological properties.

Materials & Methods: Three Southern Russian soil types, namely Haplic Chernozem Calcic, Haplic Arenosols Eutric and Haplic Cambisols Eutric, having significantly different resilience ability to Bi contamination were studied. Bismuth contamination of these soils was simulated in the laboratory by addition to the soil as conditional permissible concentrations of Bi nitrate of 1, 10 and 100, equivalent to 3 mg/kg, 30 mg/kg and 300 mg/kg, respectively. Changes in soil biological parameters were evaluated at 10 d, 30 d and 90 d after the contamination.

Results: The bismuth-contaminated soils generally lost their biological properties. The degree of recovery of biological soil properties depended on the concentration and duration of exposure to Bi. The toxic effect of Bi was identified at 10 d after the contamination, with the peak recorded on day 30. However, the soil biological properties of the Haplic Chernozem Calcic soil began to be restored up until day 90. The order of resilience to Bi contamination for the tested soils was: Haplic Chernozem Calcic > Haplic Arenosols Eutric > Haplic Cambisols Eutric.

Main finding: The suggested regional maximum permissible concentrations can be used for soil assessment not only in Southern Russia but also in similar soils worldwide as: 8.5 mg/kg for Haplic Chernozem Calcic, 2.2 mg/kg for Haplic Arenosols Eutric and 1.8 mg/kg for Haplic Cambisols Eutric soils.

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Introduction

Soils pollution by heavy metals is a major concern globally (Zhang et al., 2011; Chae and An, 2018). Among the heavy metals, bismuth (Bi) is characterized by a low content in the Earth's crust (Kabata-Pendias, 2010). However, in modern technologies, Bi is used on a par with nitrogen, carbon and chlorine (Kasimov and Vlasov, 2012). The increased Bi content is observed in all environmental components (Dobrowolski et al., 2015): in the atmosphere (Cabrera et al., 1999; Soriano et al., 2012) and in soil (Meyer et al., 2007; Abdel-Fadeel et al., 2018). The major sources of environmental and soil contamination with Bi include metal processing industry (Cortada et al., 2018) and vehicles (Xiong et al., 2015). Furthermore, Bi containing shale ash is widely used as soil fertilizer. Ore deposits featuring Bi are responsible for increasing its background content in the soil cover up to 300 times (Yurgenson and Gorban, 2017). Increased Bi presence in soil results in its accumulation in plants (Jung et al., 2002; Wei et al., 2011). Via food chains, Bi enters the human body causing multiple pathological conditions (Li et al., 2014). However, the studies focusing on the effect of Bi on living organisms are inconsistent. A few studies have provided a growing body of evidence of the negative impact of Bi on soil enzymatic activity (Murata, 2006), soil bacteria and plants (Zhang et al., 2011), earthworms (Omouri et al., 2018) and humans (Slikkerveer and de Wolff, 1989; Liu et al., 2011; Reus et al., 2018). Nevertheless, a few studies found a stimulating effect by Bi nanoparticles on the growth of cereals (Skryabin, 2016). However, study of the effect of Bi on the biological properties of soils remains insufficient. It is very important to develop sustainable models of the impact of Bi on soil conditions in terms of the amount of Bi applied and the time after pollution. Furthermore, it seems vital to identify soil resilience to Bi contamination and to introduce maximum permissible concentrations (MPCs) for Bi in soils. In soils in Southern Russia Bi, mean values for Bi are 1.1–1.2 mg/kg, with the largest mean value (2.0–3.4 mg/kg) found in the soils of the Black Sea coastal areas (Alekseenko and Alekseenko, 2013). Several authors have reported the concentration of Bi in soils varying from 0.15–2 mg/kg (Lyapunov, 2014) to 930–1891 mg/kg (Elekes and Busuioc, 2010).

The present study focused on the effect of Bi contamination on soil biological properties. The first part of the study considered three types of soil—Haplic Chernozem Calcic, Haplic Arenosols Eutric and Haplic Cambisols Eutric—while the second part considered just one soil (Haplic Chernozem Calcic) because it plays a major role in agricultural production and is widely distributed. The aim of this study was to investigate the effect of Bi contamination in soils at different levels on some soil biological properties.

Materials and Methods

Soil sampling

A variety of soils found in Southern Russia were selected with considerably different properties regarding their resilience to heavy metal contamination: Haplic Chernozem Calcic, Haplic Arenosols Eutric and Haplic Cambisols Eutric soils (IUSS Working Group WRB, 2015). The incubation study was based on soil samples taken from the topsoil layer (0–10 cm), because major contaminants, including heavy metals, are generally held in the top layer (Kabata-Pendias, 2010). Data on soil sampling locations and a brief analysis of the soils' basic physical and chemical soil indicators are provided in Table 1.

Table 1 Description of soil sampling areas

Soil type	Sampling area	Geographical coordinates	Land type	Humus content (%)	pH	Particle size distribution
Haplic Chernozem Calcic	The Botanical Garden, Southern Federal University, Rostov-on-Don	47°14'17.54 N 39°38'33.22 E	Arable land	3.7±0.2	7.8±0.5	Heavy loam
Haplic Arenosols Eutric	Rostov Region, Ust'-Donetskiy district	47°46.015 N 40°51.700 E	Grass and cereal steppe	2.3±0.1	6.8±0.3	Sandy loam
Haplic Cambisols Eutric	Republic of Adygea, nickel settlement	44°10.649 N 40°9.469 E	Horn beam and beech forest	1.8±0.1	5.8±0.3	Heavy loam

Values are presented as mean ± SD

Incubation experiment

The incubation experiment involving each type of soil contamination by Bi was carried out in triplicate in the laboratory. The Bi values were expressed as conditional permissible concentrations (CPCs). The CPC value is determined from three background concentrations of Bi in the soil due to the detection of the toxicity of heavy metals and metalloids at three background concentrations of elements in the soil (Kolesnikov et al., 2010). The average background content of Bi in the soil is 1.1 mg/kg (Alekseenko and Alekseenko, 2013). As a result, this study adopted 1 mg/kg as the Bi CPC value. Since one CPC is equal to three backgrounds (in micrograms per kilogram), the following calculation was carried out. The effects were studied of various CPCs of Bi (1, 10 and 100) equivalent to 3 mg/kg, 30 mg/kg and 300 mg/kg, respectively). The interest in studying extremely high Bi concentrations in the was determined by the significant concentrations in areas near highways of up to 930–1891 mg/kg (Elekes and Busuioc, 2010).

Bi is usually introduced into the soil in the form of nitrate; bismuth nitrate is a highly water-soluble compound, so the Bi enters the soil in a mobile form that presents its maximum toxicity (Sudina et al., 2021). At the same time, the amount of nitrogen entering the soil is negligible compared with its initial amount in the soil (approximately 1% at the maximum application rate), as is the amount of bismuth nitrate entering the soil. The amount of nitrate ions entering the soil at the highest dose of Bi in the experiment was 0.06% of the content in $Bi(NO_3)_3$. In addition, unlike other Bi compounds, the nitrate ion is rapidly absorbed by the soil biota. In the present study, Bi nitrate was studied. Egorysheva et al. (2015) found that Bi in soils in most cases is represented in the form of trivalent compounds.

Bismuth (III) nitrate dissolved in water was introduced into the soil (1 kg) and incubated at the optimum humidity (60% of the field moisture capacity) and a temperature of 20–22°C. The focus was to study the biological soil properties as these are the most sensitive and are the first to respond to chemical exposure (Kolesnikov et al., 2000). The biological properties of soil were examined after 10 d, 30 d and 90 d of exposure to Bi contamination (Kolesnikov et al., 2020).

Soil analyses

Laboratory and analytical examinations used the conventional techniques applied in biology, soil science and ecology. To assess the state of soils after contamination with Bi, the physicochemical properties were studied before contamination (pH, organic matter content, granulometric composition) and compared with the biological properties of the soils after contamination with Bi.

Before Bi contamination, the soils were determined for soil organic matter content (%), pH and particle size distribution. The potassium dichromate oxidation method (National Agricultural Technology Extension and Service Center, 2006) was used to determine the organic matter content in the soil samples. The soil pH was measured using an electrode potentiometer in distilled water using the ratio of 1 part soil to 2.5 parts of water (weight per volume). The granulometric composition was analyzed using the hydrometer method (Lovelland and Whalley, 2000. The division of soils was according to Ditzler et al. (2020).

The evaluation of the Bi toxicity was carried out using biological methods of analysis (Table 2): total number of bacteria, *Azotobacter* sp. abundance, activity of catalase and dehydrogenases, germination rate and length of roots of radish plants. The set of biological indicators provided an informative picture of the biological processes taking place in the soil and the ecological state of the soil.

Measurement of total number of soil bacteria

The total number of bacteria in the soil reflects the state of reducers in the ecosystem. The current study determined the total number of bacteria in the soil using the luminescence microscopy method, considering the number of soil bacteria after staining with acridine orange dye. Acridine orange is a fluorochromatic dye that binds to nucleic acids bacteria and other cells and under the influence of ultraviolet radiation, acridine orange stains ribonucleic acid (RNA) and single-stranded DNA in orange color (as soil particles), double-stranded DNA in green (as bacterial cells). After incubation, the fresh soil was dried and a soil suspension (soil:water, 1:100) was prepared. On prepared glasses (defatted and sterilized), 10 µL of soil suspension was placed, air-dried (air temperature 22–24°C), and dried in a burner flame (duration 3–5 s). After that, the glasses were stained with a solution of acridine orange dye (dilution of the solution of acridine orange dye, 1:10,000) for 20 min. The glasses were washed to remove excess dye and dried in the air. The glasses were viewed under a Carl Zeiss Axio Lab A1 microscope at a magnification of ×40 (20 bacterial cells of counting fields).

Table 2 Characteristics of biological indicators of soil condition

Biological indicator (measurement unit)	Functional indicator	Method	Number of measurements
Total number of bacteria ($\times 10^9$ in 1 g soil)	Characterizes the state of reducers in the ecosystem (Kolesnikov et al., 2000)	Luminescent microscopy	*n* = 720: 3 vegetation vessels with soil × 3 soil samples × 4 square centimeters on slides × 20 fields of view
Azotobacter sp. Abundance (% fouling lumps)	Indicator of chemical pollution of the soil (Akimenko et al., 2018)	Method of fouling lumps on *Ashby* medium	*n* = 225: 3 vegetation vessels with soil × 3 soil samples in *Petri* dishes × 25 fouling lumps
Catalase activity (ml O_2 per 1 g of soil in 1 min)	Most sensitive to chemical pollution among enzymes (Kolesnikov et al., 2000)	Rate of decomposition of hydrogen peroxide	*n* = 36: 3 vegetation vessels soil in 3 biological repetitions × 4 analytical repetitions
Dehydrogenases activity (mg of triphenilformazane per 10 g of soil 24 hr)		Rate of conversion of triphenyltetrazolium chloride to triphenylformazane	*n* = 36: 3 vegetation vessels with soil in 3 biological repetitions × 4 analytical repetitions
Phytotoxicity of soils (% of control)	More sensitive indicator of toxicants in the soil (Kolesnikov et al., 2010)	Germination of radish seeds (*Raphanus sativus L.*) variety "16 days" and length of roots	*n* = 225: 3 vegetation vessels in 3 biological repetitions in Petri dishes × 25 radish seeds

Measurement of Azotobacter sp. abundance

Azotobacter sp. abundance is traditionally used to indicate chemical contamination of soils (Val'kov et al., 1997). The *Azotobacter* sp. abundance was determined by the method of fouling lumps on Ashby medium. To assess the number of bacteria, Ashby medium was prepared and poured into Petri dishes into which lumps of moistened soil (25 pieces per 1 dish, temperature of incubation, 22–25°C) were stirred. These operations were performed in an abacterial air-box (BAVnp-01—"Laminar-S"). The number of fouling lumps was counted 14 d after the start of the experiment. Counting of soil lumps overgrown with *Azotobacter* sp. abundance was carried out relative to the control.

Measurement of activity levels of catalase and dehydrogenases

The activity levels of catalase and dehydrogenases were used to estimate potential biological activity in soils. Oxidoreductases (catalase and dehydrogenases) are more sensitive to chemical contamination than other enzymes (Kolesnikov et al., 2000). Catalase activity was determined according to the Galstyan method (Galstyan, 1978). The enzyme activity was determined using the gasometrical method by the rate of decomposition of 5% hydrogen peroxide after contact with the soil (temperature, 20–22°C). Dehydrogenases were determined according to Galstyan's method modified by Khaziev (2005). The activity of dehydrogenases was determined by the conversion of Triphenyl tetrazolium chloride to Triphenyl formazane. The optical density of the colored solutions was determined spectrophotometrically on a PE 5800VI spectrophotometer at a wavelength of 540 nm.

Measurement of germination rate and length of roots of radish

Soil phytotoxicity was investigated by the germination rate of radish (*Raphanus sativus* L.) and the length of roots in a growth chamber (Binder KBW 240). To assess soil toxicity using plants, garden radish (*Raphanus sativus* L.) was used. Compared to other plant test objects, radish has a fast response to soil nutrients and moisture (Pandey, 2006). The germination rate and root length of radish are the most informative of the many indicators of soil phytotoxicity (Bab'eva and Zenova, 1989). After incubation of the soil with Bi for 30 d, the soil was placed in a Petri dish and radish seeds were planted in it—25 seeds per dish in conditions of moisture content 60% and temperature 24–25°C. After 7 d of the experiment, the radish germinates were pulled out of the soil and the germination of seeds and the length of the radish roots were determined. The germination rate was assessed based on the number of germinated radish seeds in 7 d of the experiment (after the appearance of two or more leaves).

Data analyses

Based on the above biological indicators, the integral indicator of the biological state (IIBS) of the soil was determined (Kolesnikov et al., 2000, 2019). 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 that value, the percentages in other experimental variants (in polluted soil) were expressed as a percentage. For IIBS, the maximum value of each index (100%) is chosen from array data and reference to this value of this index was expressed for other variants of experiments based on Equation 1:

$$B_1 = \frac{B_x}{B_{max}} \times 100\% \quad (1)$$

where B_1 is the relative score of the indicator, B_x is the actual value of the indicator and B_{max} is the maximum value of the indicator.

The integral index of the soil biological state was calculated using Equation 2:

$$IIBS = \frac{B}{B_{max}} \times 100\% \quad (2)$$

where B is the average estimated score of all indicators and B_{max} is the maximum estimated score of all indicators.

The methodology used allowed the integration of the relative values of different indicators, the absolute values of which cannot be integrated since they have different units of measurement.The stages for calculating regional MPCs for Bi in soils were: 1) according to the results of the calculation, IIBS presented data for each concentration of Bi for each type of soil, considering the background metal content; 2) construction of functions in relation to concentration and biological effect, where feature selection focused on the highest affinity (R the correlation coefficient tended to 1), with the logarithmic function chosen among the calculated functions, as it has the highest correlation coefficient (Table 3); and 3) the equation was used to calculate the maximum permissible content of Bi in violation of certain functions of soils: low, medium and high pollution.

The regression equation describing the dependency of IIBS on the pollutant fraction in the soil was used to estimate the Bi concentration values. These regression equations allowed the calculation of pollutant concentrations responsible for degradation of particular groups of ecosystemic soil functions.

Table 3 Regression equations describing decrease in integral indicator of biological state values from bismuth content in soil

Soil type	Regression equation
Haplic Chernozem Calcic	$y = -3.986\ln(x) + 98.549$, $R^2 = 1.00$
Haplic Cambisols Eutric	$y = -5.71\ln(x) + 94.633$, $R^2 = 1.00$
Haplic Cambisols Eutric	$y = -6.269\ln(x) + 93.702$, $R^2 = 1.00$

r^2 = coefficient of determination

Statistical analyses

Data were analyzed using analysis of variance followed by the determination of the least significant difference (LSD). Variation statistics (mean values, dispersion) were determined, the reliability of different samples was established using dispersion analysis (Student's t test) and correlation analysis (Pearson correlation coefficient, r) was calculated.
LSD was determined using Equation 3:

$$LSD = S_d \times t_s \quad (3)$$

where: S_d = significant difference and t_s = Student's coefficient

Statistical data processing was carried out using the Statistica 12.0 (https://www.statsoft.com) and Python 3.6.5 Matpolib (https://www.python.org/) packages. Significance was tested at the $p < 0.05$ level.

Results

Variation of biological indicators in soils after bismuth contamination after 10 d exposure

It was determined that Bi contamination generally resulted in deteriorating biological properties of soils in Southern Russia (Fig. 1). For the Haplic Chernozem Calcic soil, applying 10 CPC and 100 CPC of Bi produced significant decreases in: catalase activity (Fig. 1A) by 11% and 15% of the control; dehydrogenases activity (Fig. 1B) by 29% and 49% of the control; and radish root length (Fig. 1E) by 15% and 21% of the control. The total number of bacteria decreased with the introduction of all studied concentrations of 1 CPC, 10 CPC and 100 CPC by 20, 37 and 39%, respectively, relative to the control (Fig. 1C). The integral indicator of the biological state of the Haplic Chernozem Calcic soil with the introduction of Bi at 10 CPC and 100 CPC decreased by 18% and 26%, respectively (Fig. 1F). For the Haplic Cambisols Eutric soil, a toxic effect was observed even when 1 CPC of Bi nitrate was applied. Furthermore, with an increase in the concentration of Bi to 100 CPC in the soil, there was a decrease in most indicators. The maximum toxic effects were observed for the total number of bacteria of 65% of the control (Fig. 1C), for catalase activity of 39% relative to the control (Fig. 1A) and for activity of dehydrogenases of 62% relative to the control (Fig. 1B). The integral indicator of the biological state of the Haplic Cambisols Eutric soil decreased with the introduction of 1 CPC, 10 CPC or 100 CPC of Bi by 18%, 27% and 40%, respectively.

For the Haplic Arenosols Eutric soil with Bi at 10 CPC and 100 of CPC, dehydrogenases inhibition was observed by 33% and 58% relative to the control (Fig. 1B). There was a dose-effect relationship. When Bi was contaminated at 1 CPC, 10 CPC or 100 CPC in the soil, the total numbers of bacteria decreased by 5%, 43% and 46%, respectively (Fig. 1C). A decrease in catalase activity by 29% relative to the control was recorded when 100 CPC Bi was added to the Haplic Arenosols Eutric soil. When amounts at 10 CPC or 100 CPC of Bi were added to the Haplic Arenosols Eutric soil, the radish roots were inhibited by 25% and 40 % relative to the control (Fig. 1E). The integral indicator of the biological state of the Haplic Arenosols Eutric soil when applying 10 CPC or 100 CPC of Bi decreased by 21% and 39%, respectively.

A low dose (1 CPC) of Bi nitrate led to unreliable stimulation of the germination of radish seeds when applied to any of the soil types (Fig. 1D). The total numbers of bacteria were significantly reduced when applied to any of the soil types, regardless of the Bi concentration (Fig. 1C). The greatest decrease in the total number of bacteria occurred for the Haplic Cambisols Eutric soil. The introduction of 100 CPC of Bi inhibited the number of soil bacteria by 65% relative to the control. In most cases, there was a decrease in the total number of bacteria, catalase and dehydrogenases activities and the length of radish roots. The Haplic Chernozem Calcic was the most resistant to the effects of Bi addition to the soil. Comparing the soil resilience to Bi contamination, the following soil series was obtained: Haplic Chernozem Calcic (84) > Haplic Arenosols Eutric (78) > Haplic Cambisols Eutric (72). The light granulometric composition of the Haplic Arenosols Eutric soil and the acidic reaction of the Haplic Cambisols Eutrics soil (pH = 5.8), as well as the low content of organic matter (1.8 and 2.3%, respectively), contributed to the high mobility and, consequently, high ecotoxicity of Bi in these soils. Thus, it was found that Bi contamination in most cases reduced the activity of soil enzymes, phytotoxic and microbiological indicators.

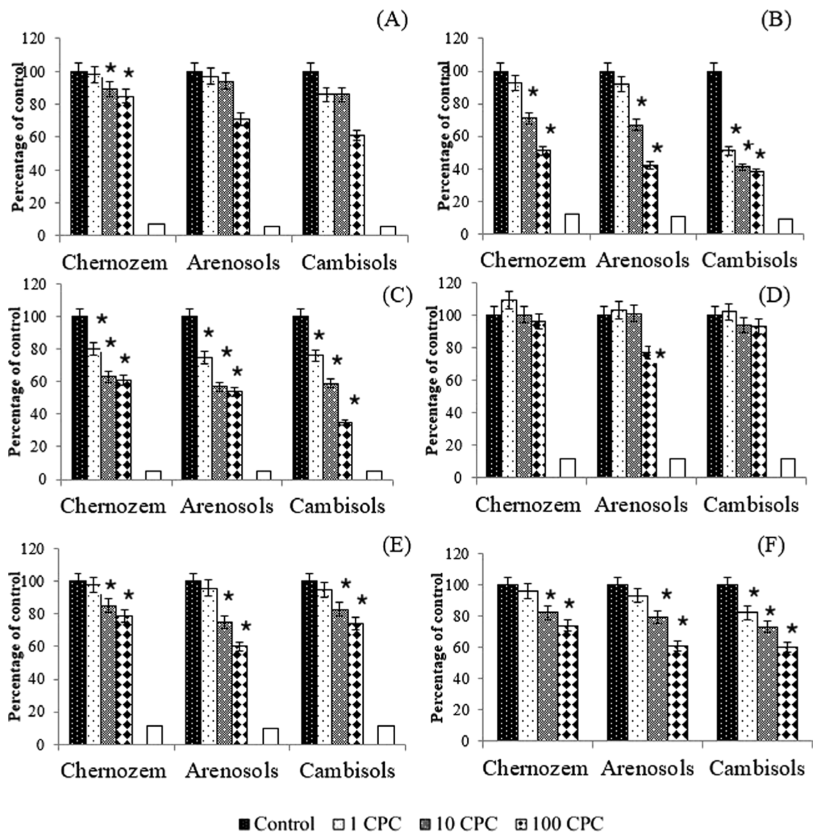

Fig. 1 Changing biological indicators in soils contaminated with bismuth found in Southern Russia after 10 d of experiment: (A) catalase activity; (B) activity of dehydrogenases; (C) total number of bacteria; (D) radish germination; (E) radish root length; (F) integral indicator of soil biological state, where CPC = conditional permissible concentration, data are mean ± SD values of three replicate biological samples and * indicates significant ($p < 0.05$) difference from control

Variation of biological indicators in Haplic Chernozem Calcic soil after Bi contamination after 10 d, 30 d and 90 d exposure

The dynamics of the changes in the biological properties from the moment of soil contamination with Bi was studied for the Haplic Chernozem Calcic soil. The most severe negative impact of Bi contamination was observed on day 30 from contamination. When assessing the dynamics of the biological state of Haplic Chernozem Calcic soil, it was noted that for most biological indicators, the strongest decrease was recorded on days 10 and 30 after contamination (Fig. 2). On day 10, the maximum decrease in dehydrogenases activity (by 49%) was observed (Fig. 2B) and for the total number of bacteria (Fig. 2C) by 39% both relative to the control. On day 30, the catalase activity compared to the control values was restored (Fig. 2C), whereas on day 30, the total number of bacteria decreased by the greatest amount (71%; Fig. 2C), the dehydrogenases activity by 67% (Fig. 2B) and the radish root length (Fig. 2E) by 32%, all relative to the control. In some cases, a non-significant stimulating effect of Bi was observed on the activities of catalase (Fig. 2A) and dehydrogenases (Fig. 2B) and the length of radish roots (Fig. 2E), but only on day 90 after contamination and only when either 1 CPC or 10 CPC of Bi had been applied. In the present study, on day 90, some biological parameters were restored to the control values, such as Azotobacter sp. abundance (Fig. 2D), or there was a tendency to restore to the control values, such as the activity of dehydrogenases (Fig. 2B), the total number of bacteria (Fig. 2C) and the length of radish roots (Fig. 2C). The lowest degree of recovery was observed for the total number of bacteria (Fig. 2C). Analysis of the dynamics based on IIBS of the Haplic Chernozem Calcic soil showed that the toxic effect of Bi was manifested already by day 10, had increased at day 30 and by day 90 the biological properties had tended to be restored (Fig. 2F).

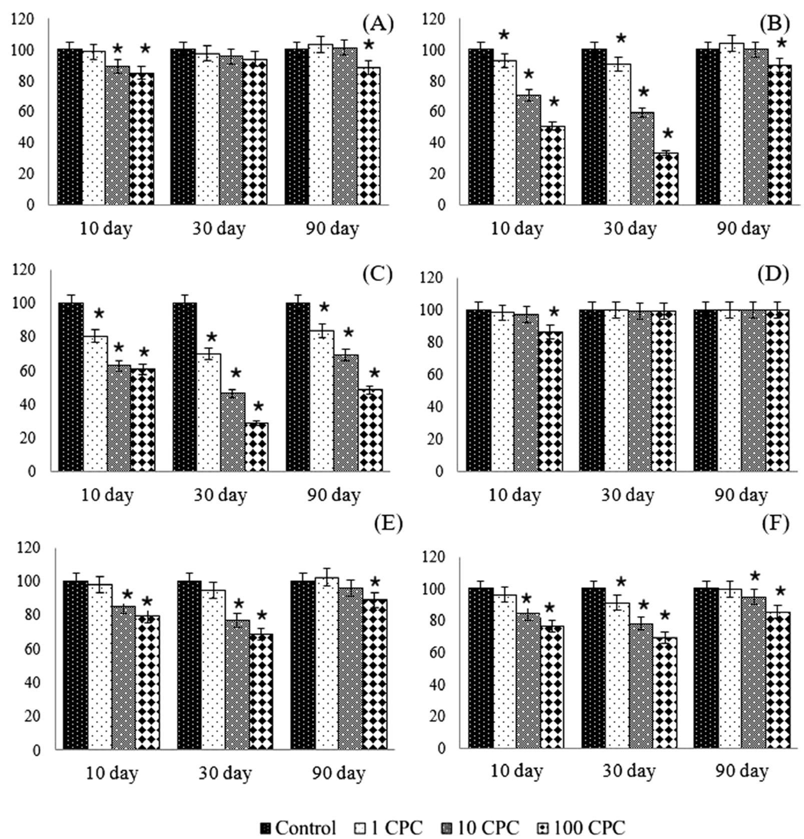

Fig. 2 Change in indicators of biological state in Haplic Chernozem Calcic after 10, 30, and 90 days of bismuth contamination, % of control: (A) catalase activity; (B) activity of dehydrogenases; (C) total number of bacteria; (D) abundance of bacterial genus *Azotobacter*; (E) radish root length; (F) integral indicator of soil biological state, where CPC = conditional permissible concentration, data are mean ± SD values of three replicate biological samples and * indicates significant ($p < 0.05$) difference from control

Assessment of bismuth contaminated Haplic Chernozem Calcic soil biological state: Sensitivity and information value

This part of the study focused on the degree of sensitivity and information value to evaluate the expedience of their application when conducting monitoring and diagnostic activities and for developing permissible standards for Bi in the environment and for soil contamination. The sensitivity degree of soil (regression equations describing the decrease in IIBS) was evaluated by correlating the value versus control samples (Table 3). Sensitivity was assessed as a percentage relative to the control (100%). The further this value was from the control value, the more sensitive the indicator. For the sensitivity rate of Bi-contaminated Haplic Chernozem Calcic soil, the biological indicators produced the following series: total number of bacteria (61) > activity of dehydrogenases (77) > radish root length (88) > catalase activity (95) > *Azotobacter* sp. abundance (98).

The information value of the indicator was evaluated according to the closeness of the correlation between the indicator and Bi concentration for the Haplic Chernozem Calcic soil (Table 3) and produced the following series: activity of dehydrogenases (-0.90) > catalase activity (-0.86) > radish root length (-0.85) > total number of bacteria (-0.75) > *Azotobacter* sp. abundance (-0.55).

The most sensitive indicators of Bi contamination in the Haplic Chernozem Calcic soil were the total number of bacteria and the activity of dehydrogenases. The most informative indicators of Bi contamination in the Haplic Chernozem Calcic was the activity of the oxidoreductases (catalase and dehydrogenases).

Discussion

When studying the effects on living organisms or soil of toxic compounds as potential toxic elements (heavy metals, non-metals, metalloids) in pesticides or antibiotics, there are cases of their stimulating effect in small amounts (Zvyagintsev et al., 1997; Kabata-Pendias, 2010). The likely cause of the inhibiting effect of Bi on the biological properties of soils, as for other heavy metals, was the inhibition of enzymes (Murata, 2006). Nagata (2015) found that high concentrations of bismuth nitrate inhibited the root growth of *Arabidopsis thaliana*, while low concentrations of bismuth had a stimulatory effect. A higher level of humus in the soil and neutral pH values of the soil solution ensured the presence of sufficient microorganisms for resilience to pollutants (Neina, 2019; Yakovets, 2021). The degree of reduction depends on the concentration of the pollutant. In most cases, there is a direct relationship between the concentration of the metal polluting the soil and the degree of deterioration of the studied soil properties (Gutiérrez et al., 2016). In the process of studying the effect of heavy metals (Hg, Cd, Pb, Cr, Cu, Zn, As) on the biological properties of a Haplic Chernozem Calcic soil, a stimulating effect was found at doses of 1 MPC and 10 MPC (Kolesnikov et al., 2008, 2009, 2022).

Kolesnikov et al. (2002) claimed that soil pollution by potential toxic elements disturbed ecosystem functions. In fact, the disturbance of these soil functions can occur either partially or entirely, depending on the pollutant concentration and following a strict order and sequence. The first indicator to be disturbed is the information functions followed by the biochemical, physical, and chemical, chemical and holistic functions, and finally, the physical ones. It seems reasonable to refer to this consistency of disturbed ecosystem soil functions when developing environmental standards for soil pollution. Soil IIBS was an objective indicator of a particular ecosystem function disturbance. It was reported that an IIBS decrease of less than 5 % did not result in any disturbance of ecosystem soil functions and that if IIBS values fall by 5–10%, this leads to disturbance of information-providing functions; a 10–25% fall causes disturbance of biochemical, physical and chemical, chemical and holistic functions, and finally, a fall of more than 25% causes degradation of physical functions (Kolesnikov et al., 2019).

Preventing the degradation of ecosystem soil functions is a critical task when developing environmental standards. Hence, an IIBS drop of more than 10% is evidence of a severe decline in soil functions. Thus, a pollutant concentration rate responsible for a 10% drop in soil IIBS could be referred to as the regional maximum permissible concentration (rMPC) for this pollutant in the soil and the pollutant level should not be allowed to exceed this standard rate.

By day 90, the biological properties of chernozem after contamination with Bi tended to be restored, but the control values (before soil contamination) were not achieved. Similar regularities in the dynamics of the biological properties of soils after pollution were obtained earlier for other heavy metals: Hg, Cd, Pb, Cr, Cu, Zn, etc. (Kolesnikov et al., 2009, 2019, 2022).

Inhibition of enzyme activity by Bi occurs because heavy metals inhibit enzymatic activity due to competition for the active centers of the enzyme with the substrate, denaturation of the enzyme protein and the formation of a monovalent bond with enzyme-substrate complexes (Vig et al., 2003). Such regulation mechanisms are associated with the structure of soils and the response of the soil environment (Benini et al., 2014; Kaya et al., 2015). Among the enzymes, oxidoreductases are considered the most sensitive to anthropogenic pollution. For example, oxidoreductases (catalase, dehydrogenases, peroxidases, polyphenoloxidases) are functionally important for the decomposition of pollutants, the transformation of organic matter and the maintenance of the metabolism of microorganisms (Kaczynski et al., 2016; Liu et al., 2017). Dehydrogenases are a type of intracellular oxidoreductases and play an essential role in the initial stages of the oxidation of soil organic matter by transferring electrons or hydrogen from substrates to acceptors; soil catalases decrease upon soil contamination with petroleum hydrocarbons and heavy metals (Stepniewska et al., 2009).

According to Table 4, if, for example, the Bi amount in the Haplic Chernozem Calcic soil does not exceed 2.5 mg/kg, soil functioning is normal. If the Bi concentration varies from 2.5 mg/kg to 8.5 mg/kg, it will disturb soil information functions. If the Bi concentration varies from 8.5 mg/kg to 350 mg/kg, then apart from information providing functions it will also disturb biochemical, physical and chemical, chemical and holistic functions. Finally, above a Bi concentration of 350 mg/kg there will be substantial degradation of soil physical functions. Clearly, it is unacceptable to disturb soil chemical, physical and chemical, biochemical and even more importantly, soil holistic functions that are responsible for soil fertility. For this reason, a Bi concentration of 8.5 mg/kg should be viewed as the MPC for Bi in Haplic Chernozem Calcic soil (the Bi rMPC). Recently, Kolesnikov et al. (2021) reported that there was greater toxicity of silver in Haplic Cambisols Eutric and Haplic Arenosols Eutric soil than for Haplic Chernozem Calcic soils, due to the acidic reaction of pH and light particle size distribution. . The rMPCs for the silver content in Haplic Chernozem Calcic, Haplic Arenosols Eutric and Haplic Cambisols Eutric soils were proposed to be 4.4 mg/kg, 0.9 mg/kg and 0.8 mg/kg, respectively.

Furthermore, Table 4 provides the most efficient techniques of soil remediation when soil is contaminated at a particular concentration of Bi. The higher the Bi concentration in the soil, the more a "radical" remediation technique should be applied. However, for example, if the Bi concentration in a Haplic Chernozem Calcic soil is less than 2.5 mg/kg and there is no evidence of ecological function failure, soil remediation is not necessary. If the Bi concentration is in the range 2.5 mg/kg to less than 8.5 mg/kg, it sufficient to apply phytoremediation techniques and washing procedures to the contaminated soil to reduce the Bi concentration to 2.5 mg/kg or lower. If the Bi concentration is in the range 8.5–350 mg/kg, it would be necessary to employ chemical land reclamation such as by introducing organic fertilizers, ion exchange resins, phosphorus fertilizers, lime and zeolites. Finally, if the Bi concentration in the soil exceeded 350 mg/kg, it would be necessary to implement a complex range of costly activities, such as removing the contaminated topsoil layer and replacing it with a new layer which is environmentally safe and valuable to agricultural plants. If the contaminated soil layer is completely removed, the soil will not receive secondary pollution through plants.

Differences in the MPC values for the considered soil types were associated with the particle size distribution, the content of organic matter and the reaction of the environment. The less organic matter, the lighter the structure of the soil and the lower the content of Bi that is necessary to have a toxic effect on the biological properties of the soil. The highest sensitivity for contamination with Bi was found in terms of the number of bacteria (61%) and the activity of dehydrogenases (77%), while the highest information content in terms of *Azotobacter* sp. abundance ($r = -0.55$) and the radish's seed germination rate ($r = -0.68$). The less organic matter, the lighter the soil structure and the less Bi is needed to produce an actual toxic effect on the biological properties of the soil. Haplic Cambisols Eutric and Haplic Cambisols Eutric soils have a light granulometric composition and humus content. In this regard, the value at which the toxic effect is observed is lower than that of Haplic Chernozem Calcic soil. Thus, the rMPC values of Bi for Haplic Chernozem Calcic, Haplic Arenosols Eutric and Haplic Cambisols Eutric soils are 8.5 mg/kg, 2.2 mg/kg and 1.8 mg/kg, respectively (Table 5). These established rMPCs

Table 4 Sensitivity and informational value assessment of bismuth contaminated Haplic Chernozem Calcic soil biological state

Indicator	Sensitivity rate (% from control)	Correlation coefficient
Total number of bacteria	61	-0.75
Azotobacter sp. abundance	98	-0.55
Catalase activity	95	-0.86
Activity of dehydrogenases	77	-0.90
Radish's seed germination rate	98	-0.68
Radish's root length	88	-0.85
IIBS	86	-0.87

*Sensitivity rate = degree of reducing biological indicator with bismuth soil contamination, with values averaged according to contamination dose and timing

Table 5 Scheme of environmental standards for bismuth contaminated soils in Southern Russia related to degree of failure of ecosystem functions

Degree of soil pollution	Not polluted	Little degree of pollution	Average degree of pollution	Strong degree of pollution
Degree of soil IIBS decline (%)	< 5	5–10	10–25	> 25
Disturbed ecosystem functions*	-	Information value	Chemical, physical and chemical, biochemical, holistic	Physical
Soil type			Bismuth concentration in soil (mg/kg)	
Haplic Chernozem Calcic	< 2.5	2.5–8.5	8.5–350	> 350
Haplic Arenosols Eutric	< 0.9	0.9–2.2	2.2–30	> 30
Haplic Cambisols Eutric	< 0.8	0.8–1.8	1.8–20	> 20
Soil remediation techniques	Non-required	Phyto remediation, washings	Chemical reclamation: introduction of organic fertilizers, ion exchange resins, phosphorus fertilizers, lime, zeolites, etc.	Full removal of contaminated layer and replacement with a new soil layer, environmentally safe and valuable for agricultural activities

IIBS = integral indicator of the biological state, with evaluation according to Kolesnikov et al. (2019)
*Classification of soil ecosystem functions according to Dobrovolsky and Nikitin (2006)

should be referred to when implementing various environmental activities, such as environmental impact assessment, soil and ecosystem monitoring practices, choosing polluted soil reclamation techniques, risk assessment of technogenic disasters and soil certification. The suggested rMPCs could be applied for soil assessment not only in Southern Russia but also in similar soils worldwide.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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