Impact of Iron, Copper, and Nickel Ions Introduced into the Soil Separately and in Various Combinations on Soil Microbiota

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Abstract—In a long-term model experiment, the abundance dynamics of soil microorganisms was studied as affected by the pollution of southern chernozem soils with various concentrations and combinations of iron, nickel, and copper ions. In the course of this study, soil microbiocenoses were plated on solid nutrient media and the following values were estimated: the total number of heterotrophic microorganisms on a meat–peptone agar and the numbers of iron-oxidizing microorganisms on a selective medium 0, 30, 90, and 210 days after the introduction of heavy metal ions into the soil. A characteristic diverse impact of heavy metal ions on soil microorganisms was established, and the degree of stability of soil microbiocenoses of the southern chernozem was revealed. Iron and copper concentrations of 10 and 50 RGB/MPC 30 days after soil contamination by individual metal ions or their combinations stimulated the growth of heterotrophic microorganisms in the soil microbiocenoses, and 90 days later the number of this microbial group decreased to the control levels and below. After 210 days, the microbiocenoses returned to a stable state. Nickel ions, introduced into the soil at a concentration of 50 MPC separately and in a number of combinations with other heavy metal ions, did not stimulate the growth of heterotrophic microorganisms. Opposing trends were observed in the abundance dynamics of iron-oxidizing microorganisms. With the exception of some model variants such as 10 and 50 MPC of copper, iron, nickel, and their combinations in various concentrations inhibited the growth of iron-oxidizing microorganisms in the first month after soil contamination. The inhibitory effect of a combination of heavy metal ions was stronger than that of individual metals. After 90 days, the numbers of iron-oxidizing microorganisms returned to the control level or even exceeded it. After 210 days, inhibition of the growth of iron-oxidizing microorganisms was observed in the microbiocenoses or their abundance corresponded to the value in the control soil sample.

Keywords: soil microbiocenoses, heterotrophic, iron-oxidizing microorganisms, heavy metals

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INTRODUCTION

In recent decades, technogenesis has become the leading process that determines the formation of the ecological and geochemical state of soils in urbanized territories (Kasimov and Nikiforova, 2004; Borodina, 2014). The intensive industrial use of natural resources caused significant changes in the distribution of some chemical elements in the surface layer of the aeration zone. First of all, this concerns heavy metals (HMs), the accumulation of high concentrations of which in the natural environment is associated with anthropogenic activity. Among the various substances polluting the environment, HMs and their compounds are distinguished by their widespread occurrence and high toxicity at concentrations significantly exceeding the background values, while many of them are highly persistent and capable of accumulation in living organisms when moving along the trophic chain (Abbas et al., 2014; Skugoreva et al., 2016; Goncharuk and Zagoskina, 2017). In soil, HMs are involved in the natural cycle of matter and are removed from it very slowly during leaching, erosion, and deflation and when HMs are consumed by plants (Su et al., 2014).

HMs are widely used in various industries; therefore, despite cleaning measures, they enter the environment with the smoke and dust of industrial enterprises and with industrial and domestic wastewater (Budykina, 2012; Ruchita et al., 2015), industrial waste, and emissions from vehicles, fertilizers, and pesticides (Nebolsin et al., 2004; Kurylenko et al., 2012; Qianrui et al., 2017), eventually accumulating in the soil (Larionov, 2013).

The majority of all the mineralization processes of organic residues are concentrated in the soil, providing for the conjugation of the biological and geological cycles. The soil is the ecological knot of the chains in the biosphere, in which the interaction of living and inanimate matter proceeds most intensively. The exchange of matter between the Earth’s crust, the hydrosphere, the atmosphere, and terrestrial organ-
isms is enclosed in the soil, among which soil microorganisms occupy an important place (Ilyin, 2012). The influence of HMs on the soil is expressed in a change its biological activity (Vodyanitskii, 2013). Under the influence of increased HM concentrations, a sharp decrease in the number of certain agronomically valuable groups of soil microorganisms (Murata et al., 2005; Sorokin et al., 2009; Hassan et al., 2013) and the activity of soil enzymes, such as dehydrogenases, catalases, ureases, amylases, invertase (Shi et al., 2008; Ofoegbu et al., 2013; Novoselova et al., 2016), was observed. These changes ultimately led to a decrease in soil fertility and crop yield (Abou-Shanab et al., 2005).

Metals inhibit the processes of mineralization and synthesis of various substances in soils, suppress the respiration of soil microorganisms and contribute to morphological changes in their reproductive organs, cause a microbostatic effect, and can act as a mutagenic factor (Friedlová, 2010; Stepanova and Eremeishvili, 2011; Fokina et al., 2015). Numerous allogorical, bacteriological, and mycological studies have shown that HMs actively affect the composition and abundance of soil microbial complexes. Under the influence of HMs, changes such as an increase or decrease in the total number of certain microorganisms, an increase or decrease in species diversity, or a change in dominant and subordinate species occur (Kolesnikov et al., 2008; Terekhova et al., 2017). Studies have shown that not only long-term, but also short-term soil contamination with HMs have a negative effect on the microbial activity of soils, significantly reducing the biodiversity and biomass of microbial communities (Hemida et al., 1997; Joynt et al., 2006). These negative processes are largely regulated by the type of soil and the amount of organic matter contained in it. The poorer the soil, the less resistant its microbial community to the action of HMs (Marfenina, 2005). It is known that microbial cenoses of chernozems are stable, while the stability of microbial communities can be influenced not only by the concentration of HMs and the duration of their action, but also by the combined effect of HM complexes (Kolesnikov et al., 2010; Fokina et al., 2015). Therefore, the problem of the impact of HMs on soil microflora requires a deep, comprehensive study for the establishment of the patterns, nature, and degree of the influence of HMs on the microbiological processes occurring in various soils.

Therefore, the goal of this study was investigation of the abundance dynamics of soil microorganisms under soil contamination with iron, nickel, and copper in different concentrations and combinations during a long-term model experiment.

**MATERIALS AND METHODS**

In the model experiment, we used soil (southern chernozem, Saratov oblast) with the following granulometric characteristics: particles >3 mm, 1.1%; <3–>1 mm, 0.8%; <1–>0.5 mm, 15.6%; <0.5–>0.25 mm, 37%; <0.25 mm, 45.5%. The soil was dried to an air-dry state, and large roots and inclusions were removed. The soil was sifted through a sieve with a hole diameter of 5 mm, and 1 kg of soil was placed in plastic containers. At the beginning of the experiment, the soil was moistened to 15% moisture content and was contaminated with solutions of HM salts (NiSO₄·7H₂O; CuSO₄) in different concentrations individually and in different combinations. Fe₂O₃ (hematite) was added to the soil in dry form. The calculation of the applied concentrations of HMs was carried out for the metal. When choosing the HM concentrations for the experimental study, we were guided by the following: for Ni(II) and Cu(II), we were guided by the MPC of mobile forms of these metals in soils (4.0 and 3.0 mg/kg of soil, respectively) (GN 2.1.7.2041-06). When choosing the Fe(II) concentrations, we took into account the values of the regional geochemical background (RGB) for Bashkortostan (2.3 mg/kg of soil) (Seregina et al., 2013), due to the fact that the MPC for Fe(II) in the soil has not been developed, and the RGB for Fe(II) for the Saratov oblast has not been established. Clean, uncontaminated soil was used as the control.

In total, 13 soil variants were studied in the experiment in two replicates (26 microbiocenoses) (Table 1). The experiment lasted seven months. During the experiment, the soil was loosened regularly (once or twice a week), and the humidity was maintained at a level of ~10–15% for the simulation of the natural water balance and aeration under laboratory conditions. The microbiological parameters of the soil were assessed before the introduction of pollutants into the soil (0 day), as well as in dynamics: 30, 90, and 210 days after the introduction of HMs.

The number of microorganisms in the soil was counted out by the method of limiting dilutions by plating soil suspensions on agar nutrient media and counting the colonies grown after 3–7 days of cultivation at a temperature of 28–30°C (Foght and Aislabie, 2005). The total number of culturable heterotrophic microorganisms (THMs) was estimated using a meat–peptone agar (MPA) (Difco) (Praktikum po mikrobiologii..., 2005). The abundance of cultured neutrophilic iron-oxidizing microorganisms was evaluated on an agar selective medium with the following composition g/L: FeSO₄·7H₂O, 5.9; (NH₄)₂SO₄, 0.5; NaNO₃, 0.5; K₂HPO₄, 0.5; MgSO₄·7H₂O, 0.5; citric acid, 10.0, sucrose, 2.0, peptone, 1.0; pH 7.0 (Granina et al., 2003; Zakharova and Parfenova, 2007). Inoculations on MPA and on a selective medium for the analysis of the number of iron-oxidizing microorganisms were made from dilutions of 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵ in several replicates. Typical colonies appeared on the surface of the selective medium, the growth of which was accompanied by the accumulation of yel-
low—orange iron oxides. As the colonies grew and developed, the medium also changed color, gradually oxidizing from light green to rusty for iron-oxidizing microorganisms.

All data on the abundance of soil microorganisms were recalculated for the air-dry samples. The experimental data were statistically processed using the Microsoft Excel 2010 software package (for Windows XP). Differences were considered significant at \( p \leq 0.05 \) (95% confidence interval).

**RESULTS AND DISCUSSION**

THMs is known to be a reliable sensitive monitoring indicator of the biological state of the soil (Sumampouw and Risjani, 2014); therefore, over the course of a long-term model experiment, we evaluated this microbiological indicator in soil microbiocenoses. The results of the assessment of the THMs 30 days after the introduction of pollutants into the soil (individual HMs and their combinations in various concentrations) are shown in Fig. 1a.

In the original clean soil, the THMs was \( 17.4 \times 10^5 \) CFU/g of soil. After 30 days the THMs in this soil decreased by about 1.5 times, which was probably due to the location of the soil microbiocenosis under the artificial conditions of a laboratory experiment. The introduction of Fe(II) into the soil at concentrations of 10 and 50 RGB stimulated the development of heterotrophic microorganisms in the microbiocenoses, and their total number in 30 days was \( 22.6 \times 10^5 \) and \( 33.4 \times 10^5 \) CFU/g of soil, respectively, which was two and three times higher than in variant C1 (control clean soil after 30 days after contamination). In soil with a concentration of 50 RGB Fe(II), the THMs after 30 days of the experiment was 1.5 times higher than in the sample with 10 RGB Fe(II). It is known that HMs in low concentrations can have a stimulating effect on the activity of soil microorganisms (Guzev and Levin, 2001; Sumampouw and Risjani, 2014). Our data also indicated the stimulation of the growth of heterotrophic microorganisms under the influence of both concentrations of Fe(II) studied 30 days after pollution. Another tendency was observed when Ni(II) was added to the soil. The THMs in the variant with the introduction of 10 MPC of Ni(II) after 30 days was \( 21.7 \times 10^5 \) CFU/g of soil, which was two times higher than in the control clean soil 30 days after contamination (see Fig. 1a). The THMs in soil with the introduction of 50 MPC of Ni(II) after 30 days was \( 10.4 \times 10^5 \) CFU/g of soil, which approximately corresponded to the value in clean soil 30 days after contamination. Thus, we found that Ni(II) in the soil at a concentration of 10 MPC stimulated the development of heterotrophic microorganisms in the soil microbiocenosis, but at high concentrations (50 MPC) it did not affect their abundance. The THMs in variant no. 6 with a combination of two HMs, 10 RGB Fe(II) and 10 MPC of Ni(II), 30 days after their introduction was \( 14.5 \times 10^5 \) CFU/g of soil, which was 1.4 times higher than in control C1 (see Fig. 1a). Similar results were found in soil variant no. 7. It is possible that the combined effect of the two metals was manifested in a way that the inhibitory effect of 50 MPC of Ni(II) prevailed over the stimulating effect of 50 RGB Fe(II). The addition of Cu(II) to the soil at a concentration of 10 and 50 MPC, as in the analogous variants with the addition of Fe(II), stimulated the development of heterotrophic microorganisms in the soil microbiocenosis, their total number after 30 days was \( 15.1 \times 10^5 \) and \( 24.5 \times 10^5 \) CFU/g of soil, respectively, which was 1.4 and 2.2 times higher than in the control soil C1 (see Fig. 1a). In soil with 50 MPC of Cu(II), the THMs after 30 days of the experiment was 1.6 times higher than in soil with 10 MPC of Cu(II). The THMs in variant no. 10 with a combination of two HMs, 10 RGB Fe(II) and 10 MPC of Cu(II), after 30 days of contamination was \( 12.0 \times 10^5 \) CFU/g of soil, which did not differ significantly from the value in the control soil C1 (see Fig. 1a). The combination of these HMs introduced into the soil at high concentrations promoted the stimulation of the THMs, which in variant no. 11 after 30 days of the experiment comprised \( 30.9 \times 10^5 \) CFU/g of soil, which was 2.8 times higher than in control C1. We found a similar result in the soil microbiocenosis with the addition of 50 RGB Fe(II). In variant no. 12 with a combination of three HMs, the THMs after 30 days of contamination was \( 14.1 \times 10^5 \) CFU/g of soil, which was 1.3 times higher than in the control soil C1 (see Fig. 1a). The combination of these HMs, introduced into the soil in increased concentrations (variant no. 13), did not have a noticeable effect on the THMs in comparison with the control C1. In soil with a lower concentration of the three HMs, the THMs after 30 days of the experiment was 1.6 times

| Variant   | HM          | Concentration |
|-----------|-------------|---------------|
| C (control) | –           | –             |
| 2         | Fe          | 10 RGB        |
| 3         | Fe          | 50 RGB        |
| 4         | Ni          | 10 MPC        |
| 5         | Ni          | 50 MPC        |
| 6         | Fe + Ni     | 10 RGB + 10 MPC |
| 7         | Fe + Ni     | 50 RGB + 50 MPC |
| 8         | Cu          | 10 MPC        |
| 9         | Cu          | 50 MPC        |
| 10        | Fe + Cu     | 10 RGB + 10 MPC |
| 11        | Fe + Cu     | 50 RGB + 50 MPC |
| 12        | Fe + Ni + Cu | 10 RGB + 10 MPC + 10 MPC |
| 13        | Fe + Ni + Cu | 50 RGB + 50 MPC + 50 MPC |
higher than in the soil with an increased HM concentration.

Thus, comparing the state of soil microbiocenoses in terms of THMs, it can be noted that reliable stimulation of the development of heterotrophic microorganisms in comparison with the control uncontaminated soil was observed 30 days after the introduction of 10 and 50 RGB Fe(II) into the soil, 50 MPC of Cu(II), and the combined introduction of Fe(II) and Cu(II) into the soil in increased concentrations. The introduction of Ni(II) had a stimulating effect only with a concentration of 10 MPC, while an increased concentration of Ni(II) equal to 50 MPC, as well as its joint introduction into the soil with other HMs, with Fe(II), Fe(II) and Cu(II), did not have a stimulating effect.

The results of evaluating the THMs 90 days after the introduction of pollutants into the soil are shown in Fig. 1b. In the control uncontaminated soil after 90 days (variant C2), the THMs was $13.6 \times 10^5$ CFU/g of soil. In comparison with the value of this indicator after 30 days, after 90 days the THMs increased by 1.2 times, but was less (1.3 times) than in the original clean soil. The THMs in variant no. 2 after 90 days was $13.7 \times 10^5$ CFU/g of soil, which corresponded to the value of this indicator in the control clean soil after 90 days. The stimulation of the THMs, which was observed in this variant 30 days after contamination, was not detected after 90 days. Compared with the value of this indicator after 30 days, after 90 days the THMs decreased by 1.6 times. The THMs in variant no. 3 after 90 days was $11.1 \times 10^5$ CFU/g of soil, which was 1.2 times lower than in the control C2 soil (Fig. 1b). Compared with the value of this indicator 30 days after contamination, the THMs in soil with 50 RGB Fe(II) after 90 days decreased by three times. Thus, if after 30 days the introduction of Fe(II) into the soil at a concentration of 10 and 50 RGB stimulated the development of soil heterotrophic microorganisms, and, moreover, a higher concentration of Fe(II) stimulated the development to a greater extent, then after 90 days the moderate suppression of the growth of heterotrophic microorganisms by iron at a concentration of 50 RGB was observed. The THMs in the soil microbiocenosis with 10 MPC of Ni(II) 90 days after contamination was $13.2 \times 10^5$ CFU/g of soil, which corresponded to the value of this indicator in the control C2 soil (Fig. 1b). Stimulation of the THMs observed in this variant 30 days after contamination was not detected after 90 days. Compared with the value of this indicator 30 days after contamination, after 90 days the THMs decreased by 1.6 times. The THMs in variant no. 5 after 90 days was $16.6 \times 10^5$ CFU/g of soil, which was 1.6 times more than in the control C2 soil. Similar tendencies were observed in soil microbiocenoses of variants no. 6 and no. 7. The THMs after 90 days was $8.7 \times 10^5$ and $7.0 \times 10^5$ CFU/g of soil, respectively, which was 1.6 and 1.9 times lower than in control C2 (see Fig. 1b). The THMs decreased in comparison with the THMs recorded after 30 days of the experiment in variants no. 6 and 7, by 1.6 and 2.1 times, respectively. Thus, the combination of Fe(II) and Ni(II) significantly inhibited the vital activity of heterotrophic microorganisms after 90 days, the inhibition was more significant than when these metals were applied to the soil separately. In the soil microbiocenosis with 10 MPC of Cu(II) after 90 days the THMs decreased to $6.0 \times 10^5$ CFU/g of soil, which was 2.3 times lower than in the control soil.
C2 (see Fig. 1b). Compared with the value of the indicator 30 days after contamination, after 90 days the THMs in this variant decreased by 2.5 times. In the soil microbiocenosis with 50 MPC of Cu(II), the decrease in the THMs was less noticeable; the THMs was 1.3 times lower than in the control C2 and 2.3 times lower than the THMs of this variant after 30 days. The THMs in variant no. 10 after 90 days was 11.0 × 10^5 CFU/g of soil, which was 1.2 times lower than the value in the control C2, and almost did not differ from the value in this variant after 30 days. The combination of iron and copper in high concentrations (variant no. 11) also led to a decrease in the THMs to 9.2 × 10^5 CFU/g of soil, which was 1.5 times lower than the value in the control soil C2 (see Fig. 1b). The stimulation of the THMs observed in this variant after 30 days was not detected after 90 days. Compared with the value of this indicator after 30 days, after 90 days the THMs decreased significantly by 9.6 times. In soil microbiocenoses no. 12 and no. 13 with three HMs, the inhibition of the development of heterotrophic microorganisms was found, their total number after 90 days was 3.0 × 10^5 and 8.5 × 10^5 CFU/g of soil, which was 4.5 and 1.6 times lower than in the control C2 soil (see Fig. 1b). The most significant suppression of the growth of heterotrophic microorganisms in comparison with other studied soil censoses was found in variant no. 12. Compared with the analogous variant after 30 days, the THMs in the soil decreased by 4.7 times.

Thus, after 90 days of the experiment, with the exception of variants with 10 RGB Fe(II) and 10 and 50 MPC of Ni(II), the inhibition of the development of heterotrophic microorganisms in soil microbiocenoses was found in comparison with the control. In many cases, there was a noticeable decrease in the THMs compared to the results of the analysis after 30 days.

After 210 days, the THMs in clean soil (variant C3), as compared to the value of this indicator after 90 days, decreased by 1.4 times. The THMs in soil microbiocenoses with 10 and 50 RGB Fe(II) was 12.1 × 10^5 and 8.0 × 10^5 CFU/g of soil, respectively (Fig. 1c), which was 1.3 times lower than in similar samples after 90 days. Thus, the decrease in the number of heterotrophic microorganisms, which was observed after 90 days with the addition of Fe(II) was preserved. When 10 and 50 MPC of Ni(II) were added to the soil, the THMs was 11.3 × 10^5 and 10.9 × 10^5 CFU/g of soil, respectively, which was close to the value in the control soil C3 (see Fig. 1c). In the variant with 10 MPC of Ni(II), THMs after 210 days was 1.2 times lower than in a similar sample after 90 days; with 50 MPC of Ni(II), it was 1.5 times lower. The decrease in the THMs observed in variant no. 4 after 90 days was also detected after 210 days. In the soil microbiocenosis of variant no. 6, the THMs after 210 days was 5.2 × 10^5 CFU/g of soil, which was 1.9 times lower than in the control C3. The inhibition of the development of the THMs observed after 90 days in this variant was preserved after 210 days. In the soil microbiocenosis of variant no. 7, the THMs was 11.4 × 10^5 CFU/g of soil, which was 1.2 times higher than in the control soil C3. Compared with the value (7.0 × 10^5 CFU/g of soil) after 90 days, the THMs was 1.6 times higher (see Fig. 1c). Thus, the combination of Fe(II) and Ni(II) after 210 days influenced the vital activity of heterotrophic microorganisms differently, depending on the concentration of HMs. A higher concentration moderately stimulated the development of heterotrophic microorganisms, while a lower concentration inhibited it. The addition of 10 MPC of Cu(II) to the soil led to an increase in the THMs to 12.7 × 10^5 CFU/g of soil, which was 2.1 times higher than after 90 days and 1.3 times higher than in the control soil C3. In variants no. 9 and no. 10, the THMs was insignificantly different from the control C3. In the soil microbiocenosis of variants no. 11 after 210 days, the THMs was 7.4 × 10^5 CFU/g of soil, which was slightly lower than in this variant after 90 days. In the soil microbiocenoses of variants no. 12 and no. 13, stimulation of the development of heterotrophic microorganisms was observed. The THMs was 15.8 × 10^5 and 11.7 × 10^5 CFU/g of soil, respectively after 210 days, which was 1.6 and 1.2 times higher than in the control C3 soil (see Fig. 1c).

Thus, after 210 days of the experiment, a significant inhibition of the development of heterotrophic microorganisms associated with the introduction of two combinations into the soil, 10 RGB Fe(II) and 10 MPC of Ni(II); 50 RGB Fe(II) and 50 MPC of Cu(II), was revealed. Significant growth stimulation in comparison with the control was found in the variant with three HMs: 10 RGB Fe(II) + 10 MPC of Ni(II) + 10 MPC of Cu(II), and moderate stimulation was observed in the soil microbiocenosis with 50 MPC of Ni(II) and its combination with 50 RGB Fe(II). In other microbiocenoses, a significant difference in comparison with the control soil after 210 days was not observed.

In addition to the THMs, we also investigated the number of iron-oxidizing microorganisms able to develop in neutral soil, taking into account that iron is one of the pollutants we have chosen for research. It is also known that, in the soil, magnetic iron minerals, including those of technogenic origin, can adsorb other HMs (Vodyanitskiy and Shoba, 2015). Iron can be oxidized by neutrophilic iron-oxidizing microorganisms, which include representatives of various groups of prokaryotes (Hedrich et al., 2011). As was shown by researchers (Pinevich, 2005; Zakharova et al., 2010), heterotrophic microorganisms oxidize Fe(II), removing in this way H₂O₂, which is formed in their metabolic processes. This function in microorganisms is manifested under specific environmental conditions.

The results of assessing the number of cultivated neutrophilic iron-oxidizing microorganisms 30 days after the introduction of pollutants into the soil are
shown in Fig. 2. In the original uncontaminated soil, the number of iron-oxidizing microorganisms was $10.4 \times 10^5$ CFU/g of soil. After 30 days the number of iron-oxidizing microorganisms in the C1 variant decreased by 1.2 times, amounting to $8.8 \times 10^5$ CFU/g of soil. Similar results were obtained when analyzing the THMs in soil after 30 days of the experiment. The introduction of 10 and 50 RGB Fe(II) into the soil significantly inhibited the development of iron-oxidizing microorganisms in the soil, their number after 30 days amounted to $3.3 \times 10^5$ CFU/g of soil in both variants, which was 2.7 times lower than in the control C1 soil. When Ni(II) was added to the soil, inhibition of the development of iron-oxidizing microorganisms was also observed, which was directly proportional to the concentration of this HMs. The number of iron-oxidizing microorganisms in the microbiocenosis with 10 MPC of Ni(II) after 30 days was $5.9 \times 10^5$ CFU/g of soil, which was 1.5 times lower than in the control C1 soil (Fig. 2a).

The content of iron-oxidizing microorganisms in soil with 50 MPC of Ni(II) after 30 days was $1.4 \times 10^5$ CFU/g of soil, which was 6.3 times lower than in control C1. The introduction of 10 RGB Fe(II) into the soil in combination with 10 MPC of Ni(II) led to a significant decrease in the number of iron-oxidizing microorganisms after 30 days by up to $0.7 \times 10^5$ CFU/g of soil, which was 14.8 times lower than in the control C1 soil (see Fig. 2a). In the microbiocenosis of variant no. 7, inhibition of the vital activity of iron-oxidizing microorganisms was also observed, the number of which after 30 days of the experiment was $1.9 \times 10^5$ CFU/g of soil, which was 4.6 times lower than in the control soil C1. As under the influence of individual HMs, the number of iron-oxidizing microorganisms was significantly reduced. It was noted that the inhibitory effect of the combination of these HMs was stronger than the effect of individual metals. The introduction of 10 and 50 MPC of Cu(II) into the soil had the opposite effect, significantly stimulating the development of iron-oxidizing microorganisms in the soil; their number after 30 days was $20.3 \times 10^5$ and $17.7 \times 10^5$ CFU/g of soil, respectively, which was 2.3 and 2 times higher than in control C1. Our results are consistent with the known literature data on the stimulating effect of low HM concentrations on the growth of soil microorganisms (Guzev and Levin, 2001; Sumampouw and Risjani, 2014). The number of iron-oxidizing microorganisms in the soil microbiocenosis of variant no. 10 after 30 days was $3.0 \times 10^5$ CFU/g of soil, which was 2.9 times lower than in the control C1 soil (see Fig. 2a). The combination of iron and copper in an increased concentration also contributed to a decrease in the number of iron-oxidizing microorganisms; after 30 days of the experiment it was $7.0 \times 10^5$ CFU/g of soil, which was 1.3 times lower than in control C1. In the soil microbiocenosis of variant no. 12 with a combination of three HMs, the number of iron-oxidizing microorganisms after 30 days of the experiment was $1.5 \times 10^5$ CFU/g of soil, which was 5.9 times lower than in the control soil C1. The addition of iron, nickel, and copper to the soil at increased concentrations reduced the content of iron-oxidizing microorganisms to $0.5 \times 10^5$ CFU/g of soil, which was 17.6 times lower than in the C1 control. The combination of Fe(II), Ni(II), and Cu(II) markedly inhibited the vital activity of iron-oxidizing microorganisms after 30 days, more than these metals introduced into the soil separately. The most significant suppression of
the growth of iron-oxidizing microorganisms after 30 days of the experiment in comparison with other studied soil cenoses was found in variant no. 13.

The analysis of soil microbiocenoses in terms of the number of iron-oxidizing microorganisms showed that the stimulation of the development of iron-oxidizing microorganisms in comparison with the control uncontaminated soil after 30 days of the experiment occurred only when 10 and 50 MPC of Cu(II) were introduced into the soil. All other HMs and their combinations at different concentrations had an inhibitory effect.

After 90 days of the experiment, the number of iron-oxidizing microorganisms in the microbiocenosis with 10 RGB Fe(II) was $5.1 \times 10^5$ CFU/g of soil, which was slightly lower than in the control soil after 90 days (variant C2) (see Fig. 2b). Compared with the value of the previous analysis (after 30 days), the number of iron-oxidizing microorganisms in this microbiocenosis has increased. On the contrary, the number of iron-oxidizing microorganisms in soil with 50 RGB Fe(II) after 90 days was $1.6 \times 10^5$ CFU/g of soil, which was 2.7 times lower than in the control C2 soil and two times lower than in this variant after 30 days. Thus, if after 30 days the introduction of Fe(II) into the soil at a concentration of 10 and 50 RGB inhibited the development of iron-oxidizing microorganisms equally, after 90 days, the opposite effects were observed. Further inhibition of the development of iron-oxidizing microorganisms with an increase in the exposure time was observed only in the variant with a high concentration of Fe(II). The number of iron-oxidizing microorganisms in soil microbiocenoses of variants no. 4 and no. 5 after 90 days was $9.3 \times 10^5$ and $12.2 \times 10^5$ CFU/g of soil, respectively, which was 2.1 and 2.7 times higher than in the control soil C2 (see Fig. 2b). The observed stimulation of the development of iron-oxidizing microorganisms after 90 days was directly proportional to the concentration of Ni(II), while after 30 days of the experiment in these variants, the opposite effect was observed, the inhibition of the development of iron-oxidizing microorganisms. At the same time, a higher concentration of Ni(II) inhibited iron-oxidizing microorganisms to a greater extent. The number of iron-oxidizing microorganisms in the microbiocenoses of variants no. 6 and no. 7 was $5.6 \times 10^5$ and $5.0 \times 10^5$ CFU/g of soil, which was slightly higher than in control C2 (see Fig. 2b). Compared with the analysis results after 30 days of the experiment, the number of iron-oxidizing microorganisms increased in variants no. 6 and no. 7 by 8 and 2.6 times. With the introduction of 10 MPC of Cu(II) into the soil, the content of iron-oxidizing microorganisms after 90 days was at the level of $3.5 \times 10^5$ CFU/g of soil, which was slightly lower than in the control soil C2. The value of this indicator after 90 days decreased by 5.8 times compared with the result after 30 days.

The introduction of 50 MPC of Cu(II) into the soil promoted the stimulation of the number of iron-oxidizing microorganisms in comparison with the C2 control after 90 days. A comparison of the number of iron-oxidizing microorganisms in this variant after 30 days with the same indicator after 90 days, revealed that it was three times lower. In the soil microbiocenosis of variant no. 10, the number of iron-oxidizing microorganisms was $3.7 \times 10^5$ CFU/g of soil; it was approximately 1.2 times lower compared to the control C2 (see Fig. 2b) and 1.2 times higher than the number of iron-oxidizing microorganisms in this variant after 30 days. In the soil microbiocenosis of variant no. 11, the number of iron-oxidizing microorganisms was $10.9 \times 10^5$ CFU/g of soil, which was 2.4 times higher than the value in the control C2. In comparison with the value of this indicator after 30 days, the number of iron-oxidizing microorganisms after 90 days increased by 1.5 times. The combination of iron, nickel, and copper in a reduced concentration did not lead to significant differences in the number of iron-oxidizing microorganisms in comparison with the C2 control after 90 days. The number of iron-oxidizing microorganisms was $4.9 \times 10^5$ and $8.1 \times 10^5$ CFU/g of soil in variants no. 12 and no. 13, which was 1.1 and 1.8 times higher than in control C2 (see Fig. 2b). In comparison with the results of the analysis of similar microbiocenoses after 30 days, the number of iron-oxidizing microorganisms increased by 3.2 and 16.2 times, respectively.

Thus, the stimulation of the development of iron-oxidizing microorganisms in comparison with the control soil after 90 days was associated with the introduction of 10 and 50 MPC of Ni(II) into the soil; 50 MPC of Cu(II), as well as several combinations: 10 RGB Fe(II) + 10 MPC of Ni(II), 50 RGB Fe(II) + 50 MPC of Cu(II), and 50 RGB Fe(II) + 50 MPC of Ni(II) + 50 MPC Cu(II).

The number of iron-oxidizing microorganisms in the control soil after 210 days (variant C3) was $3.8 \times 10^5$ CFU/g of soil, which was 1.2 times lower than in variant C2 (Fig. 2c). With the introduction of 10 RGB Fe(II) into the soil, the number of iron-oxidizing microorganisms after 210 days was $18.0 \times 10^5$ CFU/g of soil, which was 4.7 times higher than in the control soil of C3 and 3.5 times more than in this variant after 90 days. In the microbiocenosis with 50 RGB Fe(II), on the contrary, after 90 days the inhibition of the number of iron-oxidizing microorganisms remained, probably indicating the toxic effect of iron at a concentration of 50 MPC on microorganisms. The number of iron-oxidizing microorganisms in soil microbiocenoses with 10 and 50 MPC of Ni(II) after 210 days was $1.6 \times 10^5$ and $1.8 \times 10^5$ CFU/g of soil, which was 5.8 and 6.8 times lower than this indicator in similar samples after 90 days and 2.4 and 2.1 times lower than in the C3 control. After 210 days in soil microbiocenoses with a combination of two metals,
the number of iron-oxidizing microorganisms was $1.2 \times 10^5$ and $2.9 \times 10^5$ CFU/g of soil in variants 6 and 7, which was 4.6 and 1.7 times lower than in these variants after 90 days (see Fig. 2c) and 3.2 and 1.3 times lower than in the C3 control. With the introduction of 10 MPC Cu(II) into the soil, the number of iron-oxidizing microorganisms after 210 days was $4.6 \times 10^5$ and $4.8 \times 10^5$ CFU/g of soil, which was 1.2 and 1.3 times higher than in the control soil C3 (see Fig. 2c). In the microbial community with 50 MPC of Cu(II), the content of iron-oxidizing microorganisms decreased by two times in comparison with the previous analysis after 90 days, amounting to $2.8 \times 10^5$ CFU/g of soil. The combination of iron and copper in the concentrations studied also contributed to a decrease in the number of iron-oxidizing microorganisms after 210 days up to $2.6 \times 10^5$ and $3.4 \times 10^5$ CFU/g of soil, which was 1.4 and 3.2 times lower than in a similar sample after 90 days. In the soil microbiocenoses of variants no. 12 and no. 13, the number of iron-oxidizing microorganisms in the soil after 210 days was $3.4 \times 10^5$ CFU/g of soil, not differing from the value in this variant after 90 days (see Fig. 2c). The only exception was microbiocenoses with 10 and 50 MPC of Cu(II), in which the proportion of iron-oxidizing microorganisms increased significantly after 30 days, but by 90 days it had decreased. After 210 days of the experiment, the proportion of iron-oxidizing organisms in microbiocenoses with the addition of HMs decreased, with the exception of the variant with 10 RGB Fe(II).

Thus, after 210 days of the model experiment in all soil microbiocenoses, with the exception of variant no. 2, inhibition of the development of iron-oxidizing microorganisms was noted in comparison with the results of the content of microorganisms of this group after 90 days. A reduced quantity of iron-oxidizing microorganisms was found compared with the number of iron-oxidizing microorganisms in the control soil after 210 days in most cenoses.

The comparative assessment of the proportion of heterotrophic and iron-oxidizing microorganisms in soil microbiocenoses after 30, 90, and 210 days of the experiment is shown in Fig. 3. It can be seen that in the control uncontaminated soil the proportion of iron-oxidizing microorganisms decreased over time. On the contrary, in many variants of the soil contaminated with HMs, the proportion of iron-oxidizing microorganisms increased markedly after 90 days of the experiment. The only exceptions were microbiocenoses with 10 and 50 MPC of Cu(II), in which the proportion of iron-oxidizing microorganisms increased significantly after 30 days, but by 90 days it had decreased. After 210 days of the experiment, the proportion of iron-oxidizing organisms in microbiocenoses with the addition of HMs decreased, with the exception of the variant with 10 RGB Fe(II).

CONCLUSIONS

Thus, in this study, the differing nature of the influence of HMs on soil microorganisms was established and the degree of stability of soil microbiocenoses of the southern chernozem was revealed. The introduction of iron and copper in the soil at concentrations of 10 and 50 RGB/MPC, individually and in combinations, in the first month stimulated the development of heterotrophic microorganisms in the soil microbiocenoses. Nickel introduced into the soil at a concentration of 50 MPC separately and in a number of combinations with other HMs did not stimulate the development of heterotrophic microorganisms.

With an increase in the time of HM presence in the soil, the number of heterotrophic microorganisms decreased; after three months it reached the values of the control soil; in some microbiocenoses, an inhibitory effect of HMs was observed, which was especially
pronounced for a combination of pollutants. After seven months, the results of the content of heterotrophic microorganisms indicated the formation of stable microbiocenoses. In most variants, no significant difference was found in comparison with the control soil.

As was demonstrated in the experiments conducted, the changes in the group of iron-oxidizing microorganisms, in soils of Svobodniy Town (Amur oblast), were the opposite. With the exception of variants with the addition of 10 and 50 MPC of cooper, iron, nickel, and their combinations in different concentrations had an inhibitory effect on the development of iron-oxidizing microorganisms in the first month. The inhibitory effect of HMs in combination was stronger than that of the individual metals. With an increase in the time of HM presence in the soil, the number of iron-oxidizing microorganisms increased markedly, reaching maximum values after three months, recovering to the control level or exceeding it. After seven months, with one exception, inhibition of the development of iron-oxidizing microorganisms or compliance with the value in the control soil was noted.

The fluctuations revealed in the number of microorganisms of this group indicated the lability of this microbiological soil index. The tendencies revealed in the development of soil microorganisms contribute to a better understanding of the processes occurring in soils under anthropogenic pollution.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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