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The Structural and Functional State of Soil Microbiota in a Chemically Polluted Environment

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Abstract—The structural and functional diversity of the main ecological trophic groups of soil microorganisms in meadow soils of the Central Urals anthropogenically contaminated with heavy metals was studied. The increase in the total numbers of these microorganisms in technozems, in comparison with those in agrozems, is due to the higher abundance of iron-reducing, denitrifying, nitrogen-fixing, and sulfate-reducing bacteria, an increase in cellulolytic activity, and the dependence of these characteristics on the toxic load of the soil. A reductive structure of the microbial community with the predominance of *r*-strategists, which reflects earlier stages of microbiocenoses succession under soil contamination, is formed under soil pollution with heavy metals.

Keywords: ecological trophic groups of bacteria, succession rate, oligotrophic capacity, environmental pollution, heavy metals

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INTRODUCTION

With a background of environment technogenic pollution by heavy metals (HMs), the conservative properties of the soil change: the level of active acidity, exchange capacity, structure and chemical composition of components, and the structure of humic and fulvic acids, the functional groups of which are bound with HM ions (Kostina et al., 2009). This also affects the biodiversity of the ecological and trophic groups of microorganisms, the level of their metabolic activity, and their ability to adapt to pollutants (Gadd, 1993; Zagural'skaya and Zyabchenko, 1994; Blum and Eswaran, 2004; Lorenz and Kandeler, 2006; Ivshina et al., 2014). A reduction in microbiological activity due to the inhibition of physiological and biochemical processes (Bogorodskaya et al., 2012) and an increase as a result of the death of sensitive bacteria and development of resistant bacteria (Semenova et al., 2011) are possible on technogenic substrates.

The aim of this work is study of the abundance and evaluation of the structural and functional state of the main ecological and trophic groups of soil microbiota at different levels of HM contamination of the environment. It is assumed that, under conditions of long-term (decades) pollution, a stable microbiota complex is formed in soils; this complex can maintain the necessary level of biogenic exchange, ensuring the stable functioning of the herbaceous phytocenosis (Zhuikova et al., 2015).

MATERIALS AND METHODS

Characteristics of the Territory Investigated

The investigations were carried out in the area of the Nizhnii Tagil Metallurgical Combine (OAO EVRAZ NTMK) in Nizhnii Tagil, Sverdlovsk oblast (60° E, 58° N). The combine has been in operation since 1938 and is the largest source of atmospheric pollution. The main emissions are fine dust particles containing HM ions (As^{3+}, Cd^{2+}, Cr^{3+}, Cr^{6+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}), gases (CO_2, NO_2, and SO_2), and phenols and dihydrosulfides.

Soil characteristics of the sites. The soil and vegetation cover of the study area was formed in the taiga geographic zone, in a subzone of the southern taiga. According to landscape conditions and soil characteristics, two groups of soils have previously been identified: agrozems and technozems (Zhuikova et al., 2015). Agrozems are located in agrolandscapes with agro-podzolic soils at the initial stages of the sod-forming process (abandoned fallows) and are characterized by medium fertility. The exchange complex of

these soils is saturated with bases up to 57–95%, mainly with calcium. The availability of readily hydrolyzable nitrogen (2.60–5.61 mg/100 g) and mobile phosphates (3.41–49.70 mg/100 g) of these soils is low and very low. These soils are more enriched in mobile forms of potassium (11.96–418.00 mg/100 g). Technozems are located in manmade landscapes, on industrial dumps, more than 45 years old. They are young soils formed according to the burozemic and lithozem pedogeneses, which have higher fertility, are highly saturated with bases ($V > 95\%$), and have high and very high levels of exchangeable phosphorus (11.28–158.05 mg/100 g) and potassium (38.97–544.25 mg/100 g). The supply of nitrogen under the underdeveloped sod is low (4.47–5.12 mg/100 g); in the presence of sod, it is high (29.13–57.90 mg/100 g), which can contribute to more intensive development of soil microflora.

Level of chemical contamination of the soil. The chemical composition of the soil was determined in accordance with the certified methods of analysis in the accredited laboratory of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences (Accreditation Certificate no. POCC RU.0001.515630). The sampling and analysis of the soil content of Fe, Ni, Cu, Zn, Cd, and Pb was based on the methodological guidelines RD 52.18.191-89 (*Metodika vypolneniya*..., 1990) using flame atomic absorption spectrometry on the AAS Vario 6 spectrometer (Analytik Jena AG, Germany).

In the gradient of pollution, the concentrations of mobile forms of HMs (main pollutants) reach the following: for copper, 288; for lead, 23; for cadmium, 2; for zinc, 343 μg/g of soil. On the basis of the calculated pollution index Z (the sum of the HM concentrations referred to the background values in relative units), the following zones of technogenic load were identified: background zone ($Z = 1.0$ rel. units), buffer zone ($Z =$ 3.3, 6.2 rel. units), and impact zone ($Z = 22.8$, 30.0 rel. units). The names of the zones correspond to the UNEP nomenclature (Global..., 1973). The content of mobile forms of Cd, Cu, and Zn in soil in the contaminated areas is greatly in excess of the background levels: in the buffer zone it is 10, 8, and 13 times higher and in the impact zone, 78, 10, and 29 times, respectively.

Microbiological Studies

Samples of soils (30 at each site) were aseptically sampled for microbiological analysis from the top 0- to 10-cm soil layer in May 2011 and 2012. For more effective desorption of microorganisms from the surface of soil particles, the soil was pretreatment by an ultrasonic technique of the soil suspension (1 : 10, 2–5 min) with a low-frequency dispersant Soniprep 150 (MSE) (Sanyo, Japan). The total number of microorganisms was determined using the fluorochromic dye acridine orange (*Instrumental'nye metody*..., 1982). Microorganisms were counted on a fluorescent microscope Micros MC 400FP (Austria), scanning at least 30 fields of view for each sample. The numbers were recalculated according to the formula $N_{tot} = ((4 \times a \times n)/P) \times 10^{10}$, where a is the number of cells in the field of view; P is the field of view, μm^2; n is the dilution index; 4 is the area of the sample (drop), cm^2.

To determine the number of basic ecological and trophic groups of microorganisms, the plate technique and the method of limiting dilutions were used (*Metody pochvennoi*..., 1991). Counting was carried out according to the McCready table. Isolation of soil microorganisms was carried out on elective nutrient media (Zenova et al., 2002): ammonifying microorganisms were plated on beef-extract broth; denitrifying microorganisms and anaerobic nitrogen fixers (*Clostridium* spp.), on Giltay's medium; nitrate bacteria of phases I and II, on a Vinogradskii medium; sulfate-reducing, on a Postgate B medium; iron and manganese reducing, on the Bromfield medium; heterotrophs, on beef-extract agar (BEA); hydrocarbon oxidizing bacteria (HOB), on the mineral agar medium K in the vapors of a mixture of *n*-alkanes (C_{12}–C_{17}); oligotrophs, on the mineral agar medium K with no carbon source (*Katalog shtammov*..., 1994); aerobic cellulolytic microorganisms, on the Getchinson medium. The number of free-living aerobic nitrogen-fixing bacteria of the genus *Azotobacter* was taken into account by the method of fouling lumps on the Ashby medium. Cultivation of microorganisms was carried out at 28–60°C for 7–21 days.

The functional structure of the soil microorganism complex was determined according to the ratio of the number of different physiological groups. The coefficient of oligotrophic capacity (*Oc*) and the ratio of the number of microorganisms grown on the starvation agar (SA) to the number of microorganisms grown on BEA (*Oc* = SA/BEA) were calculated to analyze the community structure; the succession rate (*Sr*), i.e., the ratio of the total number of bacteria (M) counted by the luminescence method using the fluorochromic dye acridine orange to the number of bacteria grown on BEA (P) (*Sr* = M/P) was determined (Sorokina et al., 2008; Semenova et al., 2011). The countings were carried out in three- to fivefold repetition.

The statistical processing of the results was performed taking into account the arithmetic mean (M) and its error (m). The interannual variability of the number of groups of bacteria investigated was expressed in terms of the coefficient of variation (C_v). The conjugation between the characteristics was expressed through the Spearman rank correlation coefficient (R_s). The differences between the samples and the percentage of the explained variance were estimated by a single-factor variance analysis. Multiple comparisons were carried out by the Scheffe S-method. Statistical analyses were performed using the software Stat Soft, Inc., 2012.

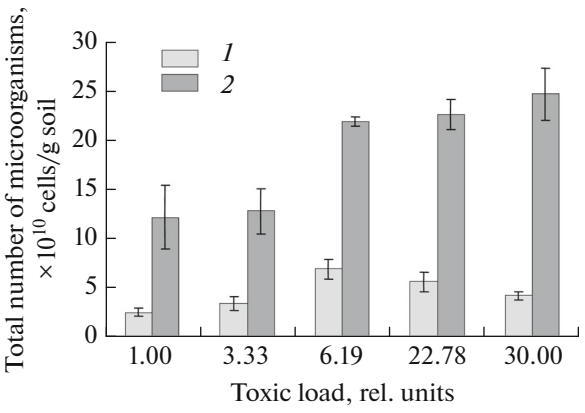

Fig. 1. Total number of microorganisms under conditions of chemical pollution (*1* is for 2011; *2*, for 2012).

RESULTS

Variations in the numbers and the ratio of ecological and trophic groups of microorganisms are among the sensitive parameters indicating a change in the state of the environment (Brooks, 1995; Chen et al., 2001; Svirskene, 2003; Polyanskaya et al., 2012; Zhao et al., 2013). Table 1 shows the quantitative characteristics of the groups of microorganisms participating in the cycles of the transformation of nitrogen, carbon, sulfur, iron, manganese, and also basic agents of cellulose destruction.

Total number of microorganisms. During the entire study period, the total number of bacteria in agrozemes soil microbiocenoses did not exceed 12.8×10^{10} cells/g of soil (Fig. 1). In technozemes this indicator was two times higher (24.4×10^{10} cells/g of soil) (*S*-method: $F(4; 11) = 3.43–5.26$, $p < 0.05–0.01$). This indicator was related to the HM soil contamination level ($R_s = 0.49–0.81$, $N = 15$, $p < 0.06–0.001$), especially in the spring–summer season of 2012. A wider variation of the total number of microorganisms during the study was determined in technozems, compared to agrozems (2.2 and 1.2–1.8 times, respectively).

Number of heterotrophic and oligotrophic bacteria. A high number of heterotrophic bacteria (5.2×10^9 cells/g of soil) was noted in 2011 in the soil of the background zone. This indicator decreases 1000 times (*S*-method: $F(4; 11) = 6.21$, $p < 0.001$) with an increase in pollution. A low level of the number of this group was observed at all the sites during the 2012 season (Table 1; $F(4; 11) = 1.56$; $p > 0.05$).

The number of oligotrophic bacteria increases in the gradient of soil pollution and is statistically significantly different in agrozones and technozems in 2011 (*S*-method: ($F(4; 11) = 5.74$, $p < 0.001$). In 2012, this indicator increased 50 times, regardless of the level of the toxic load ($R_s = 0.1$, $N = 15$; $p > 0.05$).

Number of hydrocarbon-oxidizing bacteria (HOB). In 2011, chemical pollution and the group of soils did not have a significant effect on the number of HOB ($F(4; 11) = 0.99$; $p > 0.05$). In 2012, samples from the background zone were characterized by a low number of HOB compared to the rest of the territories ($F(4; 11) = 5.04$, $p < 0.001$).

Number of bacteria participating in the nitrogen cycle. During the study a relation between the number of ammonifying bacteria and the HM soil contamination level was not revealed (2011 and 2012: $R_s = –0.25$ and 0.49, respectively, $N = 15$; $p > 0.05$); in contrast, the number of denitrifying bacteria was the lowest in the background zone and was positively correlated with the level of the toxic load regardless of the year (2011 and 2012: $R_s = 0.70–0.85$; $N = 15$; $p < 0.05–0.001$).

The abundance of nitrifying bacteria of phase I decreased in the gradient of pollution on agrozems and increased on technozems in 2011. This ecological and trophic group was not found in soils in 2012. Nitrate bacteria of phase II had an almost constant abundance at all sites (2.5×10^7 cells/g of soil) in 2012 with a slight decrease in the buffer zone (Table 1) ($R_s = 0.22$; $N = 15$; $p = 0.72$). In 2012, a sharp decrease in the number of this group of bacteria up to $10^5–10^6$ cells/g of soil on agrozems and an increase of these microorganisms up to 10^8 cells/g of soil on the technozems were observed. The dependence of the number of this group on soil pollution was statistically significant ($R_s = 0.74$, $N = 15$, $p < 0.001$).

The number of nitrogen-fixing bacteria in the soils of the background and buffer zones remained low throughout the entire study period (1–24% of the fouling lumps). In the soils of the impact zone, this indicator increased to 67–100% (*S*-method: 2011: $F(4; 75) = 10.76$; 2012: $F(4; 45) = 62.15$; $p < 0.01$) (Fig. 2). A close correlation between the ratio of bacteria of the genus *Azotobacter* and the level of pollution was revealed in both 2011 and 2012 ($R_s = 0.61–0.79$, $p < 0.001$). Significant differences in the number of nitrogen fixing bacteria on agrozems and technozems have been established (*S*-method: 2011: $F(4; 75) = 6.76$; 2012: $F(4; 45) = 12.14$; $p < 0.01$), which indicates the stability of nitrogen fixers with regard to HMs.

Number of bacteria involved in the cycles of sulfur, iron, and manganese. Sulfate-reducing bacteria were not detected in soil samples of the background zone during the study period. This indicator was low in comparison with other ecological and trophic groups of bacteria in contaminated areas (Table 1). The positive correlation between the number of these bacteria and the soil pollution level ($R_s = 0.68–0.74$, $N = 15$, $p < 0.001$) persisted during the observation period. A similar reaction to the soil contamination level was noted for iron-reducing bacteria in 2012 ($R_s = 0.59$, $N = 15$, $p < 0.001$). The manganese-reducing bacteria are the most resistant to HMs and the agrochemical composition of the soil substrate (Table 1); their number increased

Table 1. The number (cells/g soil) of ecological and trophic groups of microorganisms in the soils of the sites studied ($M \pm m$)

Ecological and trophic groups	Year of observation	Toxic load, rel. units				
		agrozems		technozems		
		1.00	3.33	6.19	22.78	30.00
Heterotrophs ($n = 3$)	2011	$(5.2 \pm 1.0) \times 10^9$	$(5.1 \pm 3.1) \times 10^6$	$(6.4 \pm 0.9) \times 10^6$	$(8.9 \pm 0.6) \times 10^6$	$(9.4 \pm 0.2) \times 10^6$
	2012	$(5.2 \pm 1.9) \times 10^6$	$(2.5 \pm 1.0) \times 10^6$	$(2.8 \pm 1.3) \times 10^6$	$(5.0 \pm 1.8) \times 10^6$	$(1.8 \pm 0.7) \times 10^7$
Oligotrophs ($n = 3$)	2011	$(3.7 \pm 0.9) \times 10^6$	$(2.1 \pm 1.3) \times 10^6$	$(1.1 \pm 0.3) \times 10^7$	$(9.5 \pm 0.3) \times 10^6$	$(10.4 \pm 0.3) \times 10^6$
	2012	$(5.9 \pm 1.9) \times 10^8$	$(2.1 \pm 0.6) \times 10^8$	$(1.7 \pm 0.1) \times 10^8$	$(3.4 \pm 1.5) \times 10^8$	$(5.1 \pm 0.5) \times 10^8$
HOB ($n = 3$)	2011	$(3.2 \pm 0.5) \times 10^9$	$(9.3 \pm 3.5) \times 10^8$	$(8.0 \pm 0.6) \times 10^7$	$(1.3 \pm 0.4) \times 10^9$	$(7.7 \pm 4.2) \times 10^8$
	2012	$(0.9 \pm 0.06) \times 10^7$	$(0.1 \pm 0.05) \times 10^9$	$(1.3 \pm 0.0) \times 10^9$	$(5.0 \pm 1.9) \times 10^8$	$(9.4 \pm 3.8) \times 10^8$
Nitrogen fixing, % ($n = 9$)	2011	0.0 ± 0.0	2.3 ± 0.7	0.0 ± 0.0	72.7 ± 11.1	66.8 ± 12.6
	2012	23.6 ± 11.3	0.7 ± 0.3	9.8 ± 3.2	97.8 ± 1.1	100.0 ± 0.0
Ammonifying ($n = 9$)	2011	$(1.8 \pm 0.4) \times 10^{10}$	$(2.7 \pm 0.6) \times 10^9$	$(1.0 \pm 0.8) \times 10^{10}$	$(2.5 \pm 0.0) \times 10^9$	$(2.5 \pm 0.0) \times 10^9$
	2012	$(1.0 \pm 0.05) \times 10^8$	$(0.2 \pm 0.1) \times 10^8$	$(9.0 \pm 3.8) \times 10^7$	$(0.2 \pm 0.04) \times 10^9$	$(0.2 \pm 0.08) \times 10^9$
Denitrifying ($n = 9$)	2011	$(9.9 \pm 7.6) \times 10^3$	$(1.9 \pm 0.7) \times 10^6$	$(8.7 \pm 2.2) \times 10^6$	$(9.3 \pm 4.8) \times 10^6$	$(1.8 \pm 0.7) \times 10^7$
	2012	$(0.2 \pm 0.02) \times 10^3$	$(0.9 \pm 0.2) \times 10^5$	$(0.2 \pm 0.07) \times 10^4$	$(4.4 \pm 0.7) \times 10^7$	$(2.3 \pm 0.4) \times 10^7$
Nitrate bacteria of phase I ($n = 3$)	2011	2.5×10^7	6.5×10^4	4.0×10^4	2.5×10^6	2.5×10^7
	2012	0.0	0.0	0.0	0.0	0.0
Nitrate bacteria of phase II ($n = 3$)	2011	2.5×10^7	9.5×10^6	2.5×10^6	2.5×10^7	2.5×10^7
	2012	$(8.0 \pm 6.0) \times 10^5$	$(3.7 \pm 0.7) \times 10^6$	$(2.0 \pm 0.3) \times 10^8$	$(2.0 \pm 0.5) \times 10^8$	$(2.0 \pm 0.5) \times 10^8$
Sulfate-reducing ($n = 9$)	2011	0.0	$(1.1 \pm 0.7) \times 10^2$	$(0.3 \pm 0.01) \times 10^2$	$(7.3 \pm 0.4) \times 10^3$	$(2.5 \pm 0.0) \times 10^2$
	2012	0.0	2.5 ± 0.0	1.8 ± 0.7	2.5 ± 0.0	2.5 ± 0.0
Iron-reducing bacteria ($n = 3$)	2011	$(2.5 \pm 0.0) \times 10^6$	$(2.5 \pm 0.0) \times 10^7$	$(2.5 \pm 0.0) \times 10^7$	$(9.5 \pm 0.0) \times 10^6$	$(9.5 \pm 0.0) \times 10^6$
	2012	$(4.7 \pm 4.1) \times 10^6$	$(0.9 \pm 0.4) \times 10^6$	$(0.1 \pm 0.06) \times 10^7$	$(1.0 \pm 0.1) \times 10^7$	$(4.7 \pm 4.1) \times 10^8$
Manganese-reduction bacteria ($n = 3$)	2011	$(2.5 \pm 0.0) \times 10^7$	$(2.5 \pm 0.0) \times 10^7$	$(2.5 \pm 0.0) \times 10^7$	$(2.5 \pm 0.0) \times 10^7$	$(2.5 \pm 0.0) \times 10^7$
	2012	$(3.1 \pm 1.4) \times 10^7$	$(1.3 \pm 0.2) \times 10^7$	$(1.3 \pm 0.6) \times 10^7$	$(11.0 \pm 0.0) \times 10^7$	$(4.5 \pm 0.0) \times 10^7$
Cellulolytic aerobic bacteria ($n = 9$)	2011	$(1.8 \pm 0.4) \times 10^4$	$(1.9 \pm 0.6) \times 10^4$	$(2.6 \pm 0.8) \times 10^4$	$(3.7 \pm 0.7) \times 10^4$	$(5.1 \pm 1.0) \times 10^4$
	2012	$(1.9 \pm 0.4) \times 10^4$	$(1.9 \pm 0.6) \times 10^4$	$(5.0 \pm 0.6) \times 10^4$	$(3.7 \pm 0.3) \times 10^4$	$(4.4 \pm 1.1) \times 10^4$
Cellulolytic anaerobic mesophilic bacteria ($n = 9$)	2011	–	–	–	–	–
	2012	$(5.5 \pm 2.1) \times 10^2$	$(9.5 \pm 0.0) \times 10^2$	$(9.0 \pm 3.0) \times 10^4$	$(3.8 \pm 0.6) \times 10^5$	$(4.0 \pm 1.0) \times 10^4$
Cellulolytic anaerobic thermophilic bacteria ($n = 9$)	2011	–	–	–	–	–
	2012	$(2.8 \pm 1.3) \times 10^3$	$(3.2 \pm 0.7) \times 10^3$	$(3.4 \pm 1.0) \times 10^4$	$(9.5 \pm 0.0) \times 10^3$	$(6.4 \pm 3.0) \times 10^3$
Microscopic fungi ($n = 3$)	2011	–	–	–	–	–
	2012	$(2.4 \pm 1.1) \times 10^6$	$(4.1 \pm 0.2) \times 10^6$	$(7.3 \pm 3.0) \times 10^4$	$(8.5 \pm 4.5) \times 10^3$	$(2.4 \pm 0.6) \times 10^5$

n is the sample size; M, the arithmetic mean value; m, the error of the mean; and the dash indicates an absence of data.

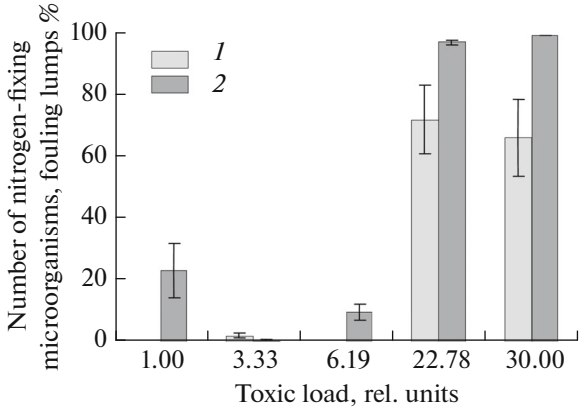

Fig. 2. Number of nitrogen-fixing microorganisms along the gradient of chemical pollution (*1* is for 2011; *2*, for 2012).

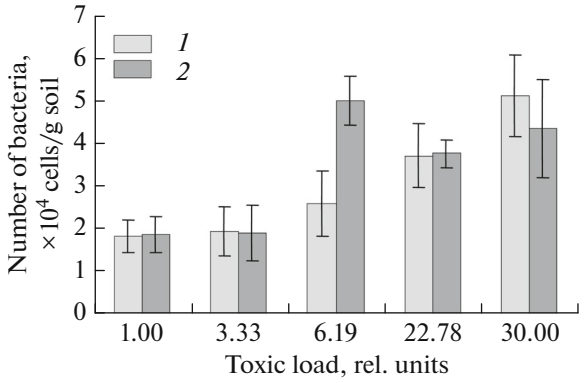

Fig. 3. Number of aerobic cellulolytic microorganisms at the sites studied (*1* is for 2011; *2*, for 2012).

insignificantly in the impact zone in 2012 in comparison to the other areas (*S*-method: $F(4; 11) = 9.6$; $p < 0.01$).

Cellulolytic bacteria. Many authors noted inhibition of the organic matter destruction rates with soil contamination by HMs and sulfur compounds (Giller et al., 1998; Vorobeichik, 2007; etc.); it is caused by a decrease in the number of bacteria of this group and the suppression of their activity (Evdokimova et al., 2013). A direct dependence of aerobic and anaerobic cellulolytic bacteria on the HM soil contamination level was detected (aerobes: $R_s = 0.65–0.74$, anaerobes: mesophylic bacteria, $R_s = 0.79$; thermophilic bacteria, $R_s = 0.60$; $N = 15$, $p < 0.05–0.001$) (Table 1; Fig. 3).

Microscopic fungi. The formation of biologically active substances in soils is also influenced by saprotrophic microscopic fungi (*Metody...*, 1991); their number in agrozems compared to technozems was 10–1000 times higher (*S*-method: $F(4; 11) = 5.68$; $p < 0.01$). The dependence of the indicator on the soil contamination level was inverse: $R_s = -0.25$; $N = 15$; $p < 0.001$.

DISCUSSION

Many authors emphasize the high lability and dependence of the abundance of various groups of bacteria (ammonifying, nitrogen-fixing, nitrifying, oligotrophic, cellulolytic) and microscopic fungi on external factors, including the presence of HMs (Gadd, 1993; Zagural'skaya and Zyabchenko, 1994; Brooks, 1995; Artamonova, 2002; Polyanskaya, 2012; Stepanov et al., 2012). On the one hand, a decrease in the number and activity of nitrifying bacteria has been observed during the action of Pb, Cu, and Ni (Brooks, 1995; Semenova et al., 2011; Evdokimova et al., 2013). On the other hand, the stability of ammonifying, denitrifying, and nitrifying bacteria to soil contamination with HMs was also emphasized (Umarov and Azieva, 1980; Zvyagintsev et al., 1997). The reason for this may be the use by these bacteria of the energy material of dead microorganisms that are highly sensitive to toxicants.

Bacteria stable to HMs are the most tolerant of other unfavorable environmental conditions. The results of the study show that the abundance of ammonifying, denitrifying, oligotrophic, and heterotrophic bacteria in the soil of the most polluted area is lower than in the background zone in different years ($C_v = 41–107$ and 118–196%, respectively).

The microbiological activity of the soil may be more correlated with its physical and chemical properties than with the HM level (Zvyagintsev et al., 1997). Our data were obtained for agrozems and technozems influenced by the different levels of HM contamination. The total number of bacteria of individual ecological and trophic groups (heterotrophs, oligotrophs, and nitrifying bacteria) differs significantly in different years. One of the reasons for this may be their reaction to weather conditions.

Among the weather and climate factors potentially affecting the development of soil microbiota, the sum of precipitation, effective temperatures, and the average monthly air temperature were considered (Table 2). The spring months in 2012 were warmer than in 2011: the average monthly air temperature in April was 3.0°C higher and the sum of effective temperatures was ten times higher. The amount of precipitation during the winter period, taking into account the precipitation in April and in the first half of May in 2012, was five times more than in 2011.

The interannual variation of the total number of organisms in soil microbiocenoses was more in the impact zone compared to the background zone and slightly contaminated area of the buffer zone (2.2 and 1.2–1.8 times, respectively). The combined effect of unfavorable weather conditions and chemical pollution leads to a decrease in the total number of soil microorganisms, especially in the impact zone.

On the other hand, oligotrophic, heterotrophic, and HOB were more sensitive to interannual fluctuations in weather conditions in the background zone

Table 2. Characteristics of the climatic and weather conditions in the region of study

Characteristics	Month (days)	Year of observation	
		2011	2012
Average temperature of the air, °C	April (1.04–30.04)	3.9	7.0
	May (1.05–15.05)	8.0	8.7
Effective temperature sum (>10°C), °C	April (1.04–30.04)	12.4	116.0
	May (1.05–15.05)	64.5	90.0
Precipitation amount, mm	Winter period (1.11–30.04)	154.1	152.8
	April (1.04–30.04)	15.8	78.7
	May (1.05–15.05)	6.3	19.7
Precipitation amount for the period with temperature >10°C, mm	April (1.04–30.04)	0	0
	May (1.05–15.05)	0	15.5

compared to the polluted environments. In the wetter and warmer year, the heterotrophs in the background zone were sharply reduced to the level of the most polluted site. This indicator even increased in the impact zone. So, for slightly heterotrophic microorganisms, slightly humidified soil is the most favorable.

Such a response to the weather conditions and soil chemical contamination was revealed for HOB; their number in the soil of the background zone differed significantly from all other sites in 2012. The opposite reaction to weather conditions and chemical pollution was demonstrated by oligotrophic bacteria that were abundant in all areas during the warm and humid spring period. The total number of microorganisms of this group decreased in the year with unfavorable weather conditions. Most likely, this group of microorganisms in the background zone was more sensitive to weather fluctuations: warm and humid conditions contribute to the development of oligotrophic bacteria, but are unfavorable for the development of heterotrophic bacteria and HOB.

The lower moisture content of the soil and the air temperature were more favorable for ammonifying and denitrifying bacteria. The bacteria of the background and buffer zones were most sensitive to these parameters.

Low variability of the number of ammonifying, denitrifying, oligotrophic, and heterotrophic bacteria is typical for most contaminated areas. High doses of HMs neutralize the effect of weather factors, which is consistent with the studies by M.M. Umarov and E.E. Aziyeva (1980). Tolerance to metals, possibly, is accompanied by the formation of resistance to unfavorable weather conditions. The increased soil moisture in our case led to an increase in the number of nitrogen-fixing bacteria, especially in contaminated sites. There is a different reaction to the HMs of soil-dependent nitrification, which depends on the weather.

Thus, the weather and climate conditions in the spring season of 2012 turned out to be more favorable for nitrogen-fixing, nitrifying, and manganese- and iron-reducing bacteria. The high moisture content in the soil and the large number of warm days in April contributed to an increase in the number of nitrogen-fixing bacteria at all the sites and to the number of iron-reducing, manganese-reducing, and nitrifying bacteria only in the highly contaminated sites. In the background and buffer zones, even a decrease in the number of the latter was observed.

Such multifactor conditions (chemical pollution, agrochemical indicators of soils, weather, and climate conditions) inevitably complicate the complex assessment of responses of microbicocenoses to technogenic effects. We can only talk about a higher overall abundance of microorganisms on technozems compared to agrozems due to an increase in the number of iron-reducing, denitrifying, nitrogen-fixing, and sulfate-reducing bacteria and an increased cellulolytic activity, as well as about the dependence of these parameters on the level of toxic load. Suppression of the activity of saprotrophic fungi was also noted.

The oligotrophic capacity and succession rate could be considered as integral indicators of the functional structure of the community of microorganisms (Sorokina et al., 2008; Semenova et al., 2011; Table 3). The difference in these coefficients in different years of observations (*Oc*: $F(1; 29) = 34.40$; *Sr*: $F(1; 29) = 66.76$; $p < 0.001$) indicates that the bacteria of the so-called "microbial pool," which are capable of increasing the activity and the number of generations in favorable conditions, occur along with actively functioning groups of microorganisms. In the conditions of high active temperatures and a sufficient degree of moistening (optimal weather conditions for most microorganisms), not only activation of heterotrophic and oligotrophic bacteria, but also an increase in the number of bacterial generations was possible. This led to an increase in the oligotrophic capacity and succession

Table 3. Characteristics (*Oc*) and (*Sr*) in the soils polluted with HMs

Characteristic	Year of observation	Toxic load, rel. units				
		agrozems		technozems		
		1.0	3.3	6.2	22.8	30.0
Oligotrophic capacity ($Oc \times 10^6$)	2011	0.001	0.41	1.72	1.07	1.11
	2012	114.8	82.0	59.4	68.7	28.3
Succession rate ($Sr \times 10^9$)	2011	4.8	6800.0	10781.3	6404.5	4468.1
	2012	23269.2	51200.0	78214.3	45200.0	13555.6

rate, especially in the background zone, in the more favorable weather conditions in 2012. Thus, the "microbial pool" ensures the maintenance of the homeostatic state of the soil, i.e., constancy of its indicative chemical and biological parameters.

If we assume that the oligotrophic capacity reflects a measure of the development of the trophic structure of the microbial community, then chemical contamination leads to a simplification of the structure of microbiocenoses, which is most pronounced on technozems (Table 3). If this reaction is nonspecific and manifests itself in response to the influence of any pessimal factors, the low *Oc* values in 2011 confirmed the less favorable weather conditions for the development of microbial communities in this year.

The differences in soils were also manifested in the values of the succession rate, which increases on agrozems and decreases on technozems with an increase in chemical pollution. The high *Sr* values in the buffer zones probably reflect the later stages of development of microbiocenoses with a predominance of microorganisms with the *K*-strategy. The low value of *Sr* at the most polluted site indicates the increasing role of rapidly growing species with the *r*-strategy, and therefore, the earlier stages of succession of the microbiocenosis. This is consistent with the data obtained earlier on the succession stages of the development of herbaceous phytocenoses in these areas (Zhuikova et al., 2015). The ruderal communities of the impact zone ($Z = 22.8–30.0$ rel. units) with a predominance of perennial grasses are characterized by a low level of projective cover and species richness, which reflects the early stage of development of their succession (the stage of secondary succession before the meadows stage) and the effect of chemical pollution.

One-way analysis of variance of the results obtained in different years of observation made it possible to estimate the contribution to the general dispersion of the two most important factors in our case: "toxic load" and "group of soils" (Table 4). The high-value effect of the toxic load on *Oc* and *Sr*, maximum for the succession rate, has been shown. The effect of the soil substrate was less significant for both indicators.

Thus, high levels of chemical pollution and unfavorable weather and climate factors contribute to the formation of microbial communities, in which the ratio of species with the *K*-strategy is reduced and the ratio of *r*-strategists is increased. This indicates a nonspecific reaction of the microbial community to stress factors.

CONCLUSIONS

(1) The effect of soil contamination by HMs on the structure of the microbial community is expressed in a change in the number and the ratio of ecological and trophic groups of microorganisms. Against the background zone, a high total number is achieved due to oligotrophic and nitrogen-fixing bacteria, at the most polluted sites, due to heterotrophic, oligotrophic, nitrogen-fixing, nitrifying, and iron-reducing microorganisms.

(2) The simplified structure of the microbial community with a predominance of *r*-strategists is formed with the highest total abundance of microorganisms in the pollution gradient in technozems; this indicates the early stages of succession of microbiocenosis.

(3) The oligotrophic capacity and succession rate of microbial communities are affected more by the level of soil contamination with HMs than by the soil group.

(4) Manganese-reducing, denitrifying, and cellulolytic aerobic bacteria are more resistant to fluctuations in the weather conditions. High soil moisture promotes an increase in the number of oligotrophic, nitrogen-fixing, nitrifying, and iron-reducing bacteria in the most polluted sites and a decrease in heterotrophic, ammonifying, nitrifying, hydrocarbon oxidizing, and iron-reducing bacteria in the soils of the background zone and, in some cases, of the buffer zones.

Thus, the high capacity for self-regulation in combination with the excess biomass of soil microorganisms and the diversity of their ecological and trophic groups contributes to the stable functioning of herbaceous phytocenoses in the gradient of soil contamination with HMs. The results of studies carried out in the southern taiga zone of the Urals characterize the state of soil microbiocenoses of specific herbaceous communities formed on specific groups of soils and influenced by a specific chemical composition and the strength of the HM pollution. This requires a careful

Table 4. Assessment of the influence of the soil group and chemical pollution on *Oc* and *Sr*

Study period	Factor	*F*	*df*	*p*	Percentage of explained variance, %
Percentage of explained variance (*Oc*)					
2011	*1*	13.11	1; 14	0.003	52.2
	2	4.36	4; 14	0.03	63.5
2012	*1*	11.90	1; 14	0.004	42.8
	2	7.01	4; 14	0.006	73.7
Percentage of explained variance (*Sr*)					
2011	*1*	4.37	1; 14	0.057	25.1
	2	24.78	4; 14	0.0001	90.8
2012	*1*	0.40	1; 14	0.54	3.0
	2	47.50	4; 14	0.0001	95.0

1 is the soil group; *2*, toxic load.

approach to direct extrapolation of the data obtained to other ecotoxicological situations.

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