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Soil Microbiocenosis as an Indicator of Stability of Meadow Communities in the Environment Polluted with Heavy Metals

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Abstract—The soil microbiota, a key component of natural ecosystems, is considered as a factor determining the stability of meadow communities. The diversity and abundance of the main ecologically significant groups of microorganisms in meadow soils have been studied along a gradient of long-term soil pollution with heavy metals in the Middle Urals. The results provide evidence for stability of the microbial assemblage formed in these soils. It has been found that the functional activity of certain physiological groups of microorganisms (nitrogen-fixing, denitrifying, and cellulolytic bacteria) and the respiratory activity of microbial communities are stimulated under conditions of heavy-metal soil pollution. Probable effects of the observed changes on mineralization of plant remains in meadow communities are discussed.

Keywords: soil, microbial communities, succession coefficient, coefficient of oligotrophy, respiratory activity, mineralization of plant remains, heavy metals, industrial pollution

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The sustainable functioning of natural biocenoses is known to largely depend on the stability of biogenic turnover of chemical elements, including organic matter mineralization in the soil. Microbiocenoses play a key role in these processes. A combined impact of physical, chemical, and biological environmental factors may result in destabilization and eventual loss of the initial microbiological properties of the soil, with consequent changes in the survival strategy of microorganisms, the development of pathogenic properties in free-living forms, and the increasing growth of parasitic and pathogenic microflora (Artamonova, 2002).

In recent years, Russian researchers have shown increasing interest in various aspects of ecomicrobiological monitoring of the environment, including situations of its chemical pollution. It should be noted that changes in soil microbiological processes caused by anthropogenic factors are difficult to distinguish from the natural dynamics of these processes, because, in any case, they involve the replacement of a certain group of actively functioning microorganisms by another group (Khaziev, 2011). Moreover, the functional activity of the soil microbiota may also change due to other factors, namely, weather–climatic conditions, the ratio of the main nutrient elements (C, N, P), the level of chemical pollution of the environment, etc. (Parshina, 2007; Pomazkina, 2011).

In the Middle Urals, numerous industries concentrated in economically developed regions are the main source of air, soil, and plant cover pollution with heavy metals, polycyclic aromatic hydrocarbons, and other highly toxic agents. Chemical pollution has a strong impact on the soil microbiota, thereby largely affecting the composition, structure, and sustainable functioning of natural biogeocenoses.

The purpose of this study was to analyze the diversity of the main ecologically significant groups of microorganisms in the Middle Urals meadow soils under conditions of long-term pollution with heavy metals and to evaluate the effect of microbiota on the rate of mineralization of plant remains in meadow communities.

MATERIAL AND METHODS

Studies were performed in the zone exposed to emissions from the Nizhny Tagil Iron and Steel Works

Table 1. Some physicochemical parameters of soils in the study region

Parameter	Toxic load, relative units				
	1	3	6	23	30
pH_{water}	6.3	6.8	6.5	7.6	8.6
Humus, %	3.6	3.9	3.7	4.1	4.0
C_{org}, %	1.7	6.7	2.3	3.6	1.6
$N_{readily\ hydrolyzable}$ compounds, mg/100 g soil	4.6	4.8	4.5	4.1	4.5
Hygroscopic moisture, %	6.6	3.2	2.7	2.7	2.3
Mobile potassium (K_2O), mg/100 g soil	23.2	39.0	12.0	57.8	26.5
Mobile phosphorus (P_2O_5), mg/100 g soil	2.6	34.3	1.7	69.6	10.8

Table 2. Contents of heavy metal mobile forms in soil samples, $M \pm m$ (2011)

S_i, rel. units	Trace elements, μg/g soil								
	Cd^{2+}	Co^{2+}	Cr^{2+}	Cu^{2+}	Fe^{3+}	Mn^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
1	0.2 ± 0.0	6.5 ± 0.9	13.1 ± 0.8	12.6 ± 0.9	788.9 ± 50.9	291.6 ± 27.2	13.0 ± 0.9	8.1 ± 0.9	17.5 ± 1.6
3	1.3 ± 0.0	16.8 ± 0.2	20.0 ± 0.5	38.6 ± 0.6	964.5 ± 1.7	359.0 ± 9.4	18.0 ± 0.3	13.2 ± 0.2	58.1 ± 1.1
6	0.9 ± 0.1	14.5 ± 3.6	7.8 ± 1.1	101.6 ± 11.1	841.1 ± 13.2	375.2 ± 54.0	7.4 ± 1.4	38.8 ± 4.9	262.7 ± 39.6
23	1.5 ± 0.5	124.2 ± 17.8	7.1 ± 2.3	951.5 ± 236.1	–	2364.9 ± 93.5	7.8 ± 1.3	12.4 ± 3.9	391.0 ± 125.9
30	2.8 ± 0.4	–	51.9 ± 3.4	194.6 ± 6.6	2736.6 ± 85.4	–	–	–	850.4 ± 18.3

Designations: S_i, total toxic load, relative units; (–) no data; $M \pm m$, arithmetic mean and standard error, $n \geq 10$.

(OAO NMTK, Evraz Group S.A.) in Sverdlovsk oblast (58° N, 60° E) from May to October 2011. The mining and smelting industry in the Urals has more than 300-year history and is responsible for a strong technogenic impact on the surrounding areas.

Test plots were laid in secondary meadow communities on heavy metal-polluted sod-podzol loam soils with a complex organic profile, different degrees of podzolization, and fine-clod structure (with the proportion of soil particles 5–10 mm in diameter reaching 67%). Basic physicochemical parameters of these soils are listed in Table 1. The procedures of soil sampling and geobotanical description of meadow communities were considered in detail in our previous publication (Zhuikova et al., 2012). Soil sample preparation for laboratory analyses of physicochemical parameters, soil biological activity, and heavy metal contents was performed by conventional methods (*Instrumental'nye metody...*, 1982; Khavezov and Tsalev, 1983; *Praktikum...*, 1991). For more effective desorption of microorganisms from the surface of soil particles, the samples were subjected to preliminary treatment involving sonication of soil suspensions (1 : 10) for 2–5 min with a Soniprep 150 ultrasonic disintegrator (MSE Ltd., United Kingdom). The rate of decomposition (mineralization) of plant remains was estimated from weight loss in the samples of dry grasses, legumes, and mixed herbage placed in nylon bags and exposed in the upper (3–4 cm) soil layer for 12 months (*Ekologicheskaya toksikologiya...*, 2001; Vorobeichik and Pishchulin, 2011).

Table 2 shows the average concentrations of heavy metals in soil samples from the test plots. As an integrated parameter of pollution, we used the index of total toxic load (S_i) calculated as the sum of the ratios of priority pollutant concentrations (Cd, Cu, Pb, Zn) in the soils of test plots to their regional background concentrations (Bezel' et al., 1998). Its values in the test plots varied from 1 to 30 relative units. Accordingly, the plots were classified as background ($S_i = 1$), buffer ($S_i = 3–6$), or impact ($S_i = 23–30$ rel. units).

Soil respiratory activity was measured with a Micro-Oxymax® respirometer (Columbus Instruments, United States); the activity of dehydrogenases involved in respiration was estimated from the degree of iodonitrotetrazolium chloride reduction. The total

count of microorganisms in soil samples was taken after acridine orange staining (*Instrumental'nye metody...*, 1982) under a Micros MC 400 fluorescence microscope (Austria). No less than 30 microscopic fields per sample were examined.

The plating technique and limiting dilution method were employed to identify and quantify representatives of different physiological groups of microorganisms, with the most probable number of microorganisms being determined using standard McCready's table. Soil samples were inoculated in (on) elective culture media (*Praktikum...*, 2002): nutrient broth for ammonifiers, Giltay medium for denitrifiers and anaerobic nitrogen fixers (*Clostridium* spp.), Winogradsky's medium for phase I and phase II nitrifiers, nutrient agar (NA) for heterotrophic microorganisms, K mineral agar medium with a mixture of *n*-alkanes (C_{12}–C_{17}) supplied through the vapor phase for hydrocarbon-oxidizing microorganisms, K mineral agar medium without a carbon source for oligotrophs (*Katalog shtammov...,* 1994), Postgate B medium for sulfate reducers, Bromfield medium for iron and manganese reducers, and Hutchinson medium for aerobic cellulolytic microorganisms. The abundance of free-living nitrogen fixers of the genus *Azotobacter* was determined on Ashby's medium using a fouling method. Microorganisms were cultured at 28–30°C for 7–21 days.

The coefficient of oligotrophy was calculated as the abundance ratio of microorganisms grown on starvation agar (SA) to those grown on NA (SA/NA), and the succession coefficient, as the ratio of the total bacterial count taken under the microscope after acridine orange staining to the number of bacteria grown on NA (Semenova et al., 2011). All counts and calculations were made in three to nine replications.

The results were processed statistically using the Statistica v. 6.0 program package. Multiple comparisons were made by Scheffe's S-method. The degree of interdependence between the test parameters was evaluated by calculating Spearman's rank correlation coefficient (R_S).

RESULTS AND DISCUSSION

According to some authors (Guzev and Levin, 2001; Giasson et al., 2010), changes in the abundance and ratio of different ecotrophic groups of microorganisms (especially those involved in the transformation cycles of nitrogen, carbon, and sulfur compounds) are the most sensitive indicator characterizing the impact of technogenic toxic substances on the soil microbiota along a pollution gradient.

As follows from Table 3, an increase in the level of meadow soil pollution with heavy metals has no significant effect on the growth of ammonifiers, phase I and phase II nitrifiers, oligotrophs, and hydrocarbon-oxidizing and iron- and manganese-reducing bacteria, whereas the total abundance of the heterotrophic microflora has a distinct tendency to decrease along the pollution gradient.

As the toxic load on soil ecosystem increases, the total abundance of soil microbiota increases as well due mainly to nitrogen fixers ($F = 275.9$; $df = 4, 11$; $p < 0.001$), denitrifiers ($F = 13.13$, $df = 4; 11$; $p < 0.001$), sulfate reducers ($F = 44.84$; $df = 4, 11$; $p < 0.001$), and aerobic cellulolytic microorganisms ($F = 8.27$; $df = 4, 11$; $p < 0.05$). This trend is directly related to the phenomenon of accelerated decomposition of plant remains in technogenically disturbed areas, which we described previously (Zhuikova et al., 2012). Differences between the values of test parameters recorded in the background zone and in polluted plots are statistically significant (Scheffe's S-method: $F = 14781.50$; $df = 4; 11$; $p < 0.001$).

It may well be that a strong and long-term technogenic impact on the studied ecosystems has promoted the development of adaptive mechanisms providing for specific tolerance of the microbial community to the toxic action of heavy metals (Giller et al., 1998). This assumption is confirmed by the high abundance (10^8–10^9 cells) of hydrocarbon-oxidizing microorganisms, which have unique metabolic properties allowing them to adapt to extreme environmental factors.

According to reported data (Val'kov et al., 1997), nitrogen-fixing bacteria of the genus *Azotobacter* are especially sensitive to heavy metals and readily respond to technogenic impact. However, we found that soils with a high level of industrial pollution (S_i = 22.78–30.00 rel. units) contained relatively large amounts of *Azotobacter*: in cultures, these bacteria formed colonies around more than 60% of soil particles. Multiple comparisons by Scheffe's S-method confirmed statistically significant differences in the abundance of nitrogen-fixing bacteria between test plots with toxic loads of 1–6 and 23–30 rel. units ($F = 275.90$; $df = 4, 11$; $p < 0.001$). A stimulating effect of heavy metal ions on nitrogen fixation processes has also been observed in model experiments by other authors (Umarova and Azieva, 1980; Zvyagintsev et al., 1997). However, it is difficult to confirm this effect in nature, under continuously changing environmental conditions.

According to our data (Fig. 1), the density of soil microbial populations in heavily polluted plots was 1.3–2.7 times higher than in background plots ($F = 55.08$; $df = 4, 146$; $p < 0.001$). It should be noted that the total abundance of microorganisms in the soil underlying the decomposing plant remains of different agrobotanical groups was not identical, being usually higher in soil below the samples of legumes. The total abundance of soil microorganisms and the density of microbial populations were found to be increased significantly in plots exposed to a greater toxic load, which could be explained by higher humus contents and a slight increase in the contents of nitrogen compounds and organic carbon in their soils (Table 1). Another relevant factor may be the change in soil pH,

Table 3. Abundance of microorganisms (cells/g soil) of different ecotrophic groups depending on toxic load on the soil of meadow communities ($M \pm m$)

Ecotrophic bacterial group	Toxic load, relative units				
	1	3	6	23	30
Total microbial abundance ($n = 30$)	$(2.5 \pm 0.7) \times 10^{10}$	$(3.4 \pm 1.2) \times 10^{10}$	$(6.9 \pm 1.7) \times 10^{10}$	$(5.7 \pm 1.4) \times 10^{10}$	$(4.2 \pm 0.7) \times 10^{10}$
Nitrogen fixers, % ($n = 15$)*	0.0	2.3 ± 0.6	0.0	72.7 ± 11.1	66.8 ± 12.6
Ammonifiers ($n = 9$)*	$(1.8 \pm 0.4) \times 10^{10}$	$(2.7 \pm 0.6) \times 10^{9}$	$(1.0 \pm 0.4) \times 10^{10}$	$(2.5 \pm 0.0) \times 10^{9}$	$(2.5 \pm 0.0) \times 10^{9}$
Denitrifiers ($n = 9$)*	$(9.9 \pm 4.4) \times 10^{3}$	$(1.7 \pm 0.8) \times 10^{6}$	$(8.7 \pm 4.7) \times 10^{6}$	$(9.3 \pm 4.5) \times 10^{6}$	$(1.8 \pm 0.4) \times 10^{7}$
Heterotrophs ($n = 3$)*	$(5.2 \pm 1.0) \times 10^{9}$	$(5.1 \pm 2.5) \times 10^{6}$	$(6.4 \pm 0.7) \times 10^{6}$	$(8.9 \pm 0.5) \times 10^{6}$	$(9.4 \pm 0.2) \times 10^{6}$
Iron-reducing bacteria ($n = 3$)	2.5×10^{6}	2.5×10^{7}	2.5×10^{7}	9.5×10^{6}	9.5×10^{6}
Manganese-reducing bacteria ($n = 3$)	2.5×10^{7}	2.5×10^{7}	2.5×10^{7}	2.5×10^{7}	2.5×10^{7}
Phase I nitrifiers ($n = 3$)	2.5×10^{7}	6.5×10^{4}	4.0×10^{4}	2.5×10^{6}	2.5×10^{7}
Phase II nitrifiers ($n = 3$)	2.5×10^{7}	9.5×10^{6}	2.5×10^{6}	2.5×10^{7}	2.5×10^{7}
Oligotrophs ($n = 3$)*	$(3.7 \pm 0.9) \times 10^{6}$	$(2.1 \pm 1.3) \times 10^{6}$	$(1.1 \pm 0.3) \times 10^{7}$	$(9.5 \pm 0.3) \times 10^{6}$	$(10.4 \pm 3.1) \times 10^{6}$
Sulfate reducers ($n = 9$)*	0.0	$(1.1 \pm 0.4) \times 10^{2}$	$(0.3 \pm 0.2) \times 10^{2}$	$(7.3 \pm 3.7) \times 10^{3}$	$(2.5 \pm 0.0) \times 10^{2}$
Hydrocarbon-reducing bacteria ($n = 3$)*	$(3.2 \pm 0.5) \times 10^{9}$	$(9.2 \pm 2.5) \times 10^{8}$	$(8.0 \pm 0.6) \times 10^{7}$	$(12.5 \pm 3.5) \times 10^{9}$	$(7.7 \pm 4.2) \times 10^{8}$
Cellulolytic bacteria ($n = 9$)*	$(1.8 \pm 0.2) \times 10^{4}$	$(1.9 \pm 0.3) \times 10^{4}$	$(2.6 \pm 0.4) \times 10^{4}$	$(3.7 \pm 0.4) \times 10^{4}$	$(5.1 \pm 0.6) \times 10^{4}$

$M \pm m$, arithmetic mean and standard error; n is the number of replications or, for the total microbial abundance, the number of microscopic fields examined.

which was found to increase from 6.3 to 8.6 along the pollution gradient. This result agrees with the data by Kovaleva et al. (2007) that an increase in the abundance of microorganisms in anthropogenically disturbed biotopes is due to alkalization of the soil environment.

Among different ecotrophic groups of microorganisms, of special interest are decomposers of organic matter contained in plant remains, aerobic cellulolytic bacteria in particular. As follows from Fig. 2, the numbers of these bacteria in the epiphytic microflora of plant remains and directly in the soil increased in plots exposed to a greater toxic load ($R_S = 0.74$; $N = 45$; $p < 0.001$). Moreover, their numbers differed depending on the kind of plant remains, being the highest below the samples of legumes and the lowest below the samples of grasses. The results of direct counts of total microorganisms and their individual groups agreed with data on soil biological activity.

Soil respiratory activity is regarded as an integrated parameter characterizing the functional activity of the broad spectrum of ecotrophic microbial groups (Krivolutskii, 1985). Its analysis in the test plots over 24 h showed that both O_2 uptake and CO_2 emission remained stable during this period but differed between the plots depending on the level of toxic load. The results of such an analysis performed in the spring and summer of 2011 are shown in Fig. 3.

The amount of CO_2 emitted from the meadow soil in the buffer and impact plots was 1.5–3.3 greater than in the background plot, reaching 2701.9–3714.3 μL/day g (Fig. 3a), with the rates of O_2 uptake and CO_2 emission varying from 0.3 ± 0.03 to 0.7 ± 0.08 and from 0.6 ± 0.01 to 2.4 ± 0.60 μL/min, respectively. It should be noted that an increase in toxic load did not result in any significant reduction of CO_2 emission, and the same was true of CO_2 uptake (Fig. 3b): the latter parameter in plots with $S_i = 3–30$ rel. units varied from 883.7 to 1355.6 μL/day g. The rates of O_2 uptake

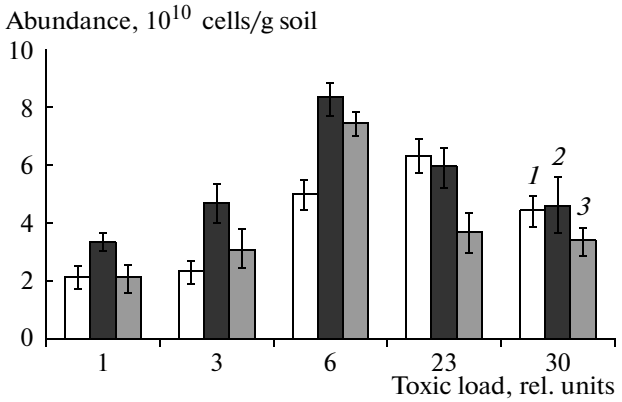

Fig. 1. Total abundance of soil microorganisms depending on toxic load. Numerals indicate samples of decomposing plant remains: (*1*) grasses, (*2*) legumes, (*3*) mixed herbage (here and in Figs. 2, 5). Error bars show errors of the mean, $n = 3$.

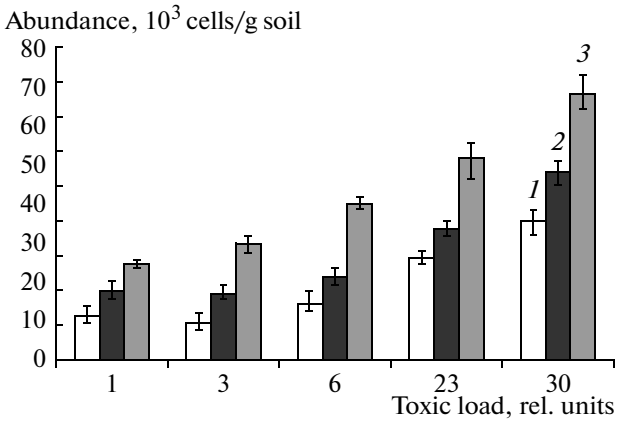

Fig. 2. Abundance of aerobic cellulolytic bacteria in the soil depending on toxic load. Error bars show errors of the mean, $n = 3$.

and CO_2 emission were found to be slightly lower in summer than in spring. This is probably explained by the fact that moisture content in the soil profile increases after spring snowmelt, which has a favorable effect on the respiratory activity of soil microorganisms.

There is an accepted opinion that the activity of soil microorganisms is suppressed under conditions of chemical pollution of the environment, which is reflected in the reduced rates of plant debris decomposition in forest and meadow communities exposed to emissions of polymetallic dust and sulfur oxides (Vorobeichik and Pishchulin, 2011). However, the effect observed in our study was the opposite: the rates of decomposition of plant remains, CO_2 emission, and O_2 uptake in meadow communities exposed to long-term technogenic impact proved to be 1.5–3.5 times higher than the background values (Fig. 4).

Soil dehydrogenase activity in samples from under the decomposing remains of legumes and grasses proved to decrease under increasing toxic load (Fig. 5). On the other hand, a significant stimulation of this activity in the soil below the remains of legumes and grasses was observed in the plot with the highest pollution level ($S_i = 30$) compared to that in other polluted plots ($S_i =$ 6–23). In all plots, soil dehydrogenase activity below the samples of decomposing mixed herbage was higher than that below other samples, but no statistically significant relationship between this activity and the level of soil pollution was revealed ($R_S = 0$–(-0.8); $N = 5$; $p > 0.1$).

Soil pollution with heavy metals also proved to have a significant effect on the coefficients of oligotrophy and succession. High values of the latter coefficient in polluted plots (4468.10–10781.30) are indicative of relatively late stages in the development of the microbial community, at which species with *K*-strategy are prevalent. The minimum value of this coefficient in the background plot (4.81) is evidence for the prevalence of fast-growing species with *r*-strategy, which is characteristic of earlier stages of succession in microbiocenosis.

The coefficient of oligotrophy varied from 0.001 in the background plot to 0.667 in the polluted plots,

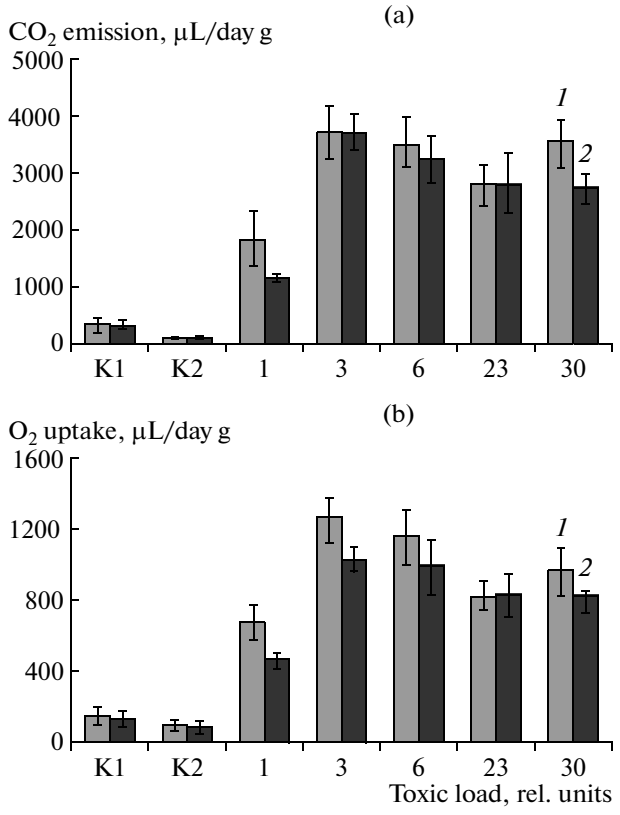

Fig. 3. Daily levels of (a) CO_2 emission and (b) O_2 uptake by soil microorganisms depending on toxic load in (*1*) May and (*2*) July 2011. K1 and K1 are model soil samples moistened with nonsterile and autoclaved distilled water, respectively. Error bars show standard deviations, $n = 9$.

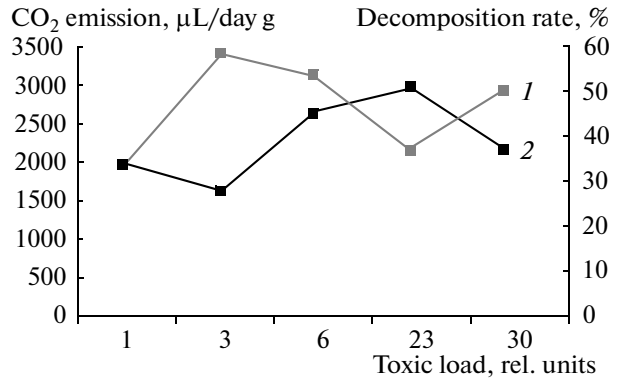

Fig. 4. Relationship between (*1*) CO_2 emission from the soil and (*2*) decomposition rate of plant remains over 12 months under conditions of technogenic soil pollution.

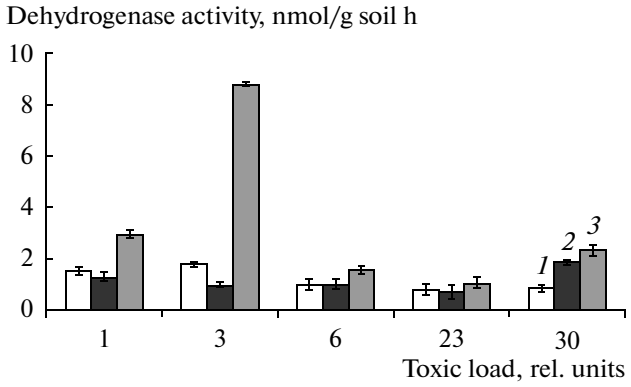

Fig. 5. Dehydrogenase activity in heavy metal-polluted soil. Error bars show standard deviations, $n = 3$.

reflecting a reduction in the stock of organic matter available to microorganisms. The low coefficient of oligotrophy in the background plot is indicative of a reduced rate of organic matter decomposition by the microbial community. This coefficient in the buffer and impact plots (S_i = 6–30) is 203 to 580 times higher, which is evidence for a high level of organic matter mineralization in soils polluted with heavy metals.

Thus, our field observations on the total abundance of soil saprotrophic microorganisms and the functional diversity of their physiological groups have provided data on changes that have occurred in the microbial community in response to long-term, increasing soil pollution with heavy metals. It has been shown that technogenic pollution of natural ecosystems in the Middle Urals has produced a stimulating effect on certain biological properties of sod-podzol meadow soils. In particular, this is manifested as an increase in the abundance of the soil microbiota as a whole (by a factor of up to 2.5) and of its individual ecotrophic groups, with the rate of mineralization of plant remains and soil respiratory activity also increasing by 24–96%. An increase in humus content (up to 4.1%) and a distinct shift of pH toward alkaline values in soils polluted with heavy metals may also stimulate the functional activity of microbial populations.

The obtained data provide evidence that mature soil microbial communities highly adapted to long-term pollution with heavy metals have developed in the studied biogeocenoses. These communities appear to be dominated by microorganisms whose life strategy is aimed primarily at their survival and successful functioning in polluted habitats rather than at accelerated growth (Zavarzin, 2011). Naturally, the qualitative and quantitative parameters of microbial communities depend not only on heavy metal contents in the soil but also on a wide range of other factors, both natural and anthropogenic. The activity of certain ecophysiological groups of microorganisms (in particular, of nitrogen fixers, denitrifiers, and cellulolytic bacteria), as well as their total abundance, account for the self-organization ability of the microbial community actively functioning in sod-podzol meadow soil for self-organization (i.e., the ability to recover normal functions and neutralize toxic agents or convert them into harmless substances). This ability, combined with the excess biomass of microorganisms and altered composition of their ecotrophic groups, not only contributes to the sustainable functioning of biogeocenoses but also improves soil resistance to technogenic pollution.

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