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Response of Soil Microbial Community to the Simultaneous Influence of Metals and an Organic Substance

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Abstract—Organic substances and metals multidirectionaly influence soil microorganisms. The results of an evaluation of the response of the soil microbial community to the simultaneous application of organic substance and metals which are a part of compost from the sewage sludge are presented. For two seasons after soil treatment, the increase in C_{org} to 2.1 ± 0.4% and that in Cd, Cr, Cu, Ni, Pb, and Zn mobile forms to 1.1 ± 0.03, 3.8 ± 0.8, 6.0 ± 1.2, 2.1 ± 0.5, 3.2 ± 0.7, and 12.3 ± 2.7 mg/kg correspondingly resulted in the growth of microbial biomass in comparison with a control soil. The respiratory activity of the treated soils increased during the first season and decreased to the level of control plots by the end of the second season. The value of a metabolic quotient did not exceed the control level. An analysis of the principal components of the obtained data revealed that the major factor determining the variability of the microbial community is the alteration of the content of organic substance in the soil.

Keywords: soil, composts, microbial biomass, respiration, metabolic quotient, metals
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INTRODUCTION

The pollution of soil by metals is mostly the result of anthropogenous activity. In some cases, toxic metals inflow in soil together with organic substances, for example, when sewage sludge and the composts of the sediments are distributed on soil (McGtath, 1995; Selivanovskaya et al., 2003; Hargreaves et al., 2008; Nwachukwu and Pulford, 2011). These organic wastes are used as a substitute for traditional organic fertilizers such as manure and, more often, for the cultivation of decorative or commercial crops.

The change in structure and functioning of the soil microbial communities under the action of both an organic substance and toxic metals has attracted much interest from researches (Vig et al., 2003; Giller et al., 1998; Giller et al., 2009; Gomes et al., 2010; Chodak et al., 2013; Tripathy et al., 2014; Kolesnikov et al., 2014; Bourioug et al., 2015), because microorganisms fulfill the processes of cycle of matter in soil and provide the fertility and productivity of soils as a whole (Schloter et al., 2003). The application of organic substance has a wholesome effect on microbial biomass and activity of soil communities, which allows us to use this method for restoring the soil quality (Nair and Ngouajio, 2012; Jannoura et al., 2014; Shi and Marschner, 2014). Metals, especially in high concentration, adversely influence the microbial activity of soils, and the greatest role belongs to the mobile fraction of metals (McGtath et al. 1995; Rost et al., 2001; Vig et al., 2003; Nwachukwu and Pulford, 2011). The extremely manifold effects of metals vary from direct action on the cells of microorganisms to the mediated action related to the change of biochemical processes in soil.

To evaluate the effects caused by anthropogenous factors, the wide spectrum of the parameters characterizing a status of microbial community is used, because only the totality of data allows us to receive an adequate evaluation. The most often used parameters are the microbial biomass evaluated by the concentration of carbon, respiration, and metabolic quotient (Vig et al., 2003; Selivanovskaya et al., 2003; Schloter et al., 2003; Niemeyer et al., 2012; Blagodatskaya and Kuzyakov, 2013; Chodak et al., 2013). Carbon of a microbial biomass (C_{mic}) is a part of the organic carbon of soil, which is a more labile indicator of the changes of environment in the case of the pollution of soil by metals than the general content of organic substance, because the change in the conditions first of and foremost results in changes in the biomass of the microorganisms of soil communities (Brookes, 1995; McGtath, 1995; Giller et al., 1998; Vig et al., 2003). The importance of this parameter is determined also by the microbial biomass stabilizing the soil ecosystems (Ouni et al., 2013). It was shown that in the soils polluted by metals of high concentrations, a microbial biomass is essentially lower than in the unpolluted soils (Barajas-Aceves, 2005; Niemeyer et al., 2012). At the same time, moderate concentrations do not significantly influence the processes (and in some cases lead to insignificant stimulation of them) (Khan and Scullion, 2002; Nwachukwu and Pulford, 2011). The intensity of the mineralization of the soil organic substance, evaluated on the basis of respiratory activity, is widely used in laboratory and field researches as an indicator sensitive to the toxic action of metals (Khan and Scullion, 2000; Rost et al., 2001; Nwachukwu and Pulford, 2011; Chodak et al., 2013). The indicator of basal respiration is often used as a criterion for the evaluation of soil fertility and reflects the availability of organic substance to soil microorganisms, because all carbon lost by soil in the course of respiration should pass through a microbial pool. A number of authors proposes the metabolic quotient (qCO_2), which is the ratio of respiratory activity to a microbial biomass, as the measure of stability of soils to various natural and anthropogenous influences, and as an indicator reflecting the disturbance and stress of soil community caused, in particular, by metals (Anderson and Domsch, 1990; Khan and Scullion, 2000; Niemeyer et al., 2012; Blagodatskaya and Kuzyakov, 2013; Tripathy et al., 2014; Spohn and Chodak, 2015). This parameter can be the most sensitive in the case of the pollution of soil by metals, because in stress conditions the microorganisms redistribute energy from growth to support maintenance.

Thus, the application of toxic metals and organic substance into soil induces multidirectional effects: the organic substance stimulates biological activity, whereas the metals negatively influence the microbial communities of soil. At the same time, the effects caused by the simultaneous inflow of organic substance and metals into soil are little-studied. In the present work, data on the response of the soil microbial community to the simultaneous inflow of metals and organic substance as part of composts from the sewage sludge in soil are presented.

MATERIALS AND METHODS

Luvisol of composition C_{org}, 0.7 ± 0.1%; N_{total}, 0.2 ± 0.03%; Zn, 15.1 ± 3.1 mg/kg; Cd, 0.4 ± 0.03 mg/kg; Ni, 24.8 ± 5.4 mg/kg; Cr, 47.1 ± 14.8 mg/kg; Cu, 31.4 ± 19.6 mg/kg; Pb, 9.9 ± 2.1 mg/kg; lay, 1%; sand, 24%; dust, 63%; and mud, 2% was used in the study. The pH value of the soil was 5.7 ± 0.1. Research was conducted on the territory of the Matjushensky wood nursery (Republic of Tatarstan, Russia).

The organic substance and metals were applied in the soil as a part of compost prepared from the sewage sludge of the Kazan station of waste water treatment.

The compost composition was as follows: C_{org}, 35 ± 6%; N_{total}, 1.7 ± 0.3%; pH, 6.1 ± 0.1; Zn, 546 ± 74 mg/kg; Cd, 12 ± 2.0 mg/kg, Ni, 77 ± 14mg/kg; Cr, 167 ± 42 mg/kg, Cu, 312 ± 45 mg/kg; and Pb, 166 ± 37 mg/kg.

The compost was applied in three doses on the plots of 2.5 × 3.5 m in size located at random in four replications. After the application, the soil was mixed at a depth of 20 cm. The quantity of the applied compost was on average 40, 62, and 75 g of C_{org}/kg of soil (variants Kl, Km, Kh, respectively). The quantity of organic substance is caused by doses of the compost application. Compost was not applied into a control soil (variant C). After the compost application, the plots were sowed with the seeds of an ordinary pine (*Pinus sylvestris*).The soil samples were selected four times during the first growing season and two times during the second growing season. The soils were selected between numbers of crops from the surface layer of 0–20 cm in depth. From each plot, the samples were selected in five replications and averaged. The sample was transported in a laboratory and stored at 4°C. Part of soil was dried to constant weight, ground, and subjected to chemical analysis. For biological analysis, the samples after storage in the refrigerator were stored at room temperature for 24 h. All analyses were made in five replications; the results were expressed per weight of dry soil.

The content of organic carbon (C_{org}) was determined by wet oxidation with 0.167 M $K_2Cr_2O_7$ followed by titration with 0.1 M $(NH_4)_2Fe(SO4)_2 \cdot 6H_2O$ (*GOST* 26213-91, 1992). The total content of metals in the soils was determined after wet oxidation of the sample with concentrated HNO_3 and 3% H_2O_2. The content of mobile forms of metals was evaluated after hourly extraction with an acetate–ammonium buffer, pH 4.8. The content of metals was evaluated by atomic absorption spectrometry using an AAnalyst-300 device (PerkinElmer, Norwalk, United States).

Carbon of the soil microbial biomass (C_{mic}) was determined using an extraction–fumigation method followed by an evaluation of the content of carbon according to ISO 14240-2 (1997). The soil basal respiration (the release of CO_2 without substratum addition) was determined according to Schinner [1995]. The metabolic quotient (qCO_2) was calculated as the ratio of the basal respiration to the soil microbial biomass (Anderson and Domsch, 1990).

The statistical analysis was performed according to R system (R Core Team, 2015). For the multivariate analysis of variance of the totality of the indicators of microbial biomass, respiratory activity, and metabolic quotient, the function aov() was used. To calculate the canonical correlation of the totality of these indicators to the content of C_{org} and to the concentrations of metals, the function cancor() was used. The part of the variance that has been explained was calculated by an analysis of variance of linear model realized by functions lm() and summary.lm(). The statistical analysis using the method of principal components was performed with the function of a vegan packet (Oksanen et al., 2014). In the figures and in the tables, average values and standard deviation are presented.

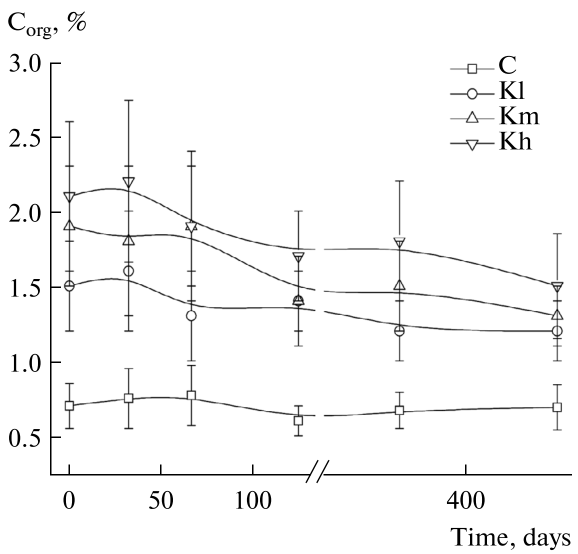

Fig. 1. Change in the content of organic carbon over the time of the experiment.

RESULTS AND DISCUSSION

The compost application into soil led to an increase in the content of C_{org}. Therefore, the content of C_{org} in the Kl, Km, Kh samples of soil, selected immediately after compost application, was 1.5 ± 0.3, 1.9 ± 0.3, and 2.1 ± 0.5%, which was above the content of C_{org} in the samples of the C variant (Fig. 1). The positive relationship between the content of organic substance in soil and the dose of the substance application was revealed. Over two growing seasons, an insignificant decrease in the content of C_{org} was observed, indicating the mineralizing activity of microorganisms. However, it is necessary to note that, by the end of the study, the content of C_{org} in the samples of experimental variants did not reach to the level of that of control variant.

One specific feature of composts from the sewage sludge is the presence of metals in the composts. Therefore, the application of the composts to increase the content of the organic substance of soil inevitably results in a simultaneous increase in the content of metals. The maximum excess (by 4.5 times) of cadmium was revealed in the samples of Kh variant when compared to the control (Table 1). The total content of other metals in the samples of this variant of study was 1.5 times above the samples of control variant. The increase in the dose of the compost led to an increase in the content of metals, excluding Cr and Pb, for which no such pronounced trend was revealed. Over the time of the experiment, no changes of the total content of metals were revealed.

The soil biota is influenced basically by mobile forms of metals. Therefore, besides the total content in soil samples, the fraction of metals extractable by the acetate–ammonium buffer was determined (Table 2). The application of composts resulted in an increase in the content of mobile forms of metals, and the value of the increase did not depend on the compost doses. In the beginning of the experiment, the maximum content of Cd, Cr, Cu, Ni, Pb, and Zn exceed the control level by 4.6, 2.4, 2.8, 1.5, 1.5, and 2.0 times, respectively. Throughout the test, practically in the samples of all variants of the experiment, the content of mobile fraction of metals was above the control variant, excluding the content of Ni in the samples of Kl and Km variants and that of Pb in the samples of Km variant.

Parallel with the analysis of the change in the characteristics of the abiotic component of the soil system, the response of the microbial community was analyzed. The total microbial biomass, respiratory activity, and metabolic quotient were characteristics of the response.

As was specified earlier, the result of applying an organic substance was for the most part an increase in the biomass and respiration of the microbial community of soil, whereas the application of metals—depending on concentration—can lead both to a decrease and an increase in respiration and, practically always, to a decrease in biomass (Brookes, 1995, McGrath et al., 1995, Giller et al., 1998, 2009; Barajas-Aceves, 2005), therefore, we will consider the received results from this point of view.

Over two growing seasons, the level of microbial biomass in the samples of the soil selected from the plates of control variant varied from 0.12 to 0.49 mg C_{mic}/g (Fig. 2). The values of the microbial biomass are comparable to the data presented in the literature. As a whole, a low enough level of the biomass such as 0.05–2.0 mg C_{mic}/g of soil was marked (McGtath et al., 1995; Bastida et al., 2008; Fernandez et al., 2009; Chodak et al., 2013; Blagodatskaya and Kuzyakov, 2013).

Table 1. Total content of metals in soils treated with compost

Variant	Content of metals, mg/kg					
	Zn	Cu	Cd	Ni	Cr	Pb
C	15.1 ± 2.0	31.4 ± 7.4	0.4 ± 0.1	24.8 ± 6.2	47.1 ± 11.2	9.9 ± 2.0
Kl	18.7 ± 3.8	54.6 ± 12.1	1.7 ± 0.3	32.1 ± 7.1	93.9 ± 24.1	15.4 ± 2.9
Km	26.2 ± 5.9	58.5 ± 15.7	1.7 ± 0.4	34.4 ± 7.8	89.8 ± 21.2	14.1 ± 3.2
Kh	28.1 ± 6.2	62.3 ± 16.5	1.9 ± 0.4	38.2 ± 9.1	96.3 ± 18.7	17.8 ± 3.8

Table 2. Content of the mobile form of metals extractable with acetate ammonium buffer in soils treated with compost

Time of the selection of samples	Variant			
	C	Kl	Km	Kh
	Cadmium, mg/kg			
0	0.1 ± 0.02	0.3 ± 0.05	0.2 ± 0.05	0.4 ± 0.07
32	0.1 ± 0.01	0.5 ± 0.1	0.5 ± 0.12	1.1 ± 0.03
66	0.2 ± 0.02	0.6 ± 0.15	0.2 ± 0.04	0.4 ± 0.05
124	0.1 ± 0.01	0.5 ± 0.1	0.5 ± 0.09	0.8 ± 0.2
360	0.2 ± 0.04	0.4 ± 0.06	0.2 ± 0.05	0.6 ± 0.15
452	0.1 ± 0.02	0.2 ± 0.04	0.1 ± 0.02	0.2 ± 0.04
	Chromium, mg/kg			
0	1.1 ± 0.03	2.0 ± 0.06	1.5 ± 0.2	2.7 ± 0.9
32	1.0 ± 0.02	2.4 ± 0.06	2.6 ± 0.4	3.5 ± 0.9
66	1.5 ± 0.03	2.0 ± 0.04	2.2 ± 0.5	3.8 ± 0.8
124	1.2 ± 0.03	2.7 ± 0.08	2.0 ± 0.4	2.4 ± 0.5
360	1.1 ± 0.02	2.3 ± 0.06	2.0 ± 0.5	2.7 ± 0.5
452	1.3 ± 0.03	3.0 ± 0.05	1.3 ± 0.3	3.8 ± 0.9
	Cooper, mg/kg			
0	1.6 ± 0.4	3.8 ± 0.9	4.6 ± 1.1	4.2 ± 0.8
32	1.5 ± 0.04	5.7 ± 1.1	5.6 ± 0.9	5.5 ± 1.2
66	2.0 ± 0.5	6.8 ± 1.4	4.2 ± 0.8	6.0 ± 1.2
124	1.7 ± 0.04	2.3 ± 0.5	4.4 ± 1.2	4.2 ± 0.7
360	1.6 ± 0.03	3.3 ± 0.3	4.2 ± 1.0	3.7 ± 0.6
452	1.5 ± 0.04	3.2 ± 0.4	4.8 ± 0.7	3.4 ± 0.4
	Nickel, mg/kg			
0	0.8 ± 0.1	0.6 ± 0.04	0.8 ± 0.07	1.2 ± 0.3
32	0.8 ± 0.2	1.0 ± 0.06	0.7 ± 0.1	1.2 ± 0.4
66	0.6 ± 0.1	0.6 ± 0.05	1.0 ± 0.2	2.1 ± 0.5
124	1.0 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
360	0.6 ± 0.1	0.7 ± 0.07	0.6 ± 0.1	0.9 ± 0.1
452	0.7 ± 0.2	0.7 ± 0.09	0.7 ± 0.15	1.2 ± 0.2
	Lead, mg/kg			
0	1.1 ± 0.2	1.7 ± 0.4	0.7 ± 0.1	0.7 ± 0.09
32	1.2 ± 0.3	2.3 ± 0.5	1.0 ± 0.2	3.2 ± 0.7
66	0.3 ± 0.04	1.4 ± 0.3	0.8 ± 0.2	2.6 ± 0.6
124	0.7 ± 0.07	1.0 ± 0.2	0.5 ± 0.41	2.0 ± 0.4
360	0.5 ± 0.06	0.7 ± 0.1	0.6 ± 0.2	1.1 ± 0.03
452	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.06	1.8 ± 0.04
	Zinc, mg/kg			
0	4.3 ± 0.8	6.0 ± 1.2	8.7 ± 1.3	7.6 ± 1.9
32	3.7 ± 0.9	8.4 ± 1.8	12.8 ± 3.2	11.0 ± 2.5
66	6.2 ± 1.5	13.5 ± 3.8	9.5 ± 2.1	12.3 ± 2.7
124	5.0 ± 1.2	11.6 ± 3.1	9.7 ± 2.0	11.7 ± 1.2
360	3.1 ± 0.9	9.2 ± 1.4	8.8 ± 1.4	8.7 ± 1.0
452	3.3 ± 0.8	6.4 ± 1.2	7.5 ± 1.1	8.5 ± 1.8

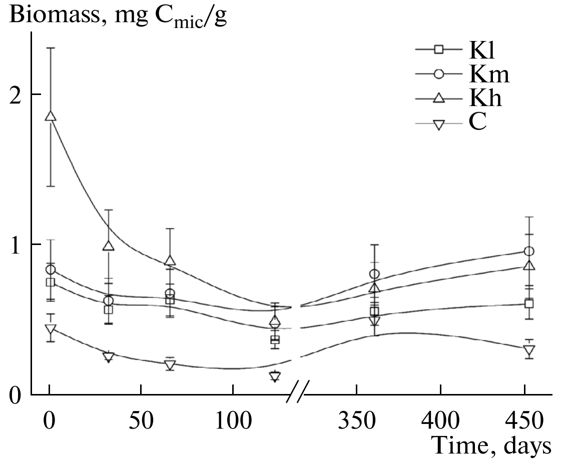

Fig. 2. Change in the level of microbial biomass over the time of the experiment.

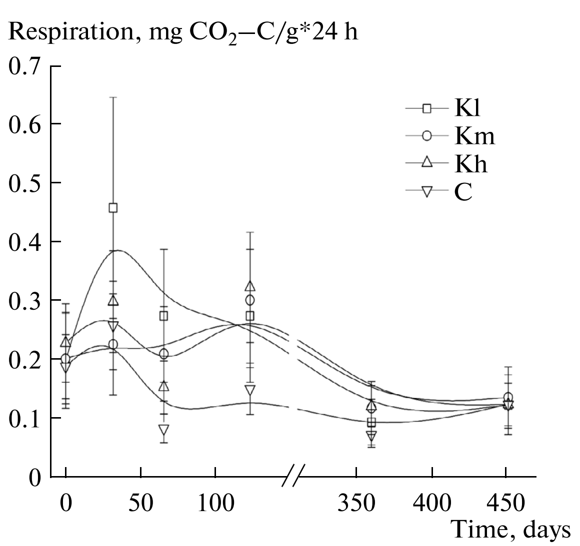

Fig. 3. Change in the respiratory activity of the soil microbial community over the time of experiment.

In the samples of all variants of the test, the application of compost led to an increase in the level of the microbial biomass of soil microbial community and the response had a dose-dependent effect (Fig. 2). The revealed dependence persisted during two growing seasons. The level of the biomass of the samples of the experimental variants authentically differed from that of the control variant. At that, the samples of various variants had maximum values of the biomass in different periods of time. Therefore, the maximum level of the biomass of the samples of the Kh variant (1.8 mg of C_{mic}/g) was noted immediately after the compost application; in the samples of the Km variant, the level of the biomass of 0.9 mg/g of C_{mic} was achieved in 452 days, and the level of the biomass of the samples of the Kl variant did not vary significantly throughout the experiment.

At the initial stage, the increase in the microbial biomass most likely can be induced, first, by the application of microorganisms as part of compost and, second, by the inflow of additional nutritious elements, the quantity of which is enough not only to increase the metabolic activity of native microflora, but also to provide growth process (Blagodatskaya and Kuzyakov, 2013). This effect is in agreement with the literature data (Moreno et al., 1999). The decrease in the level of the microbial biomass of the experimental samples during the first month is possibly related to the dieback of the introduced microflora or native microflora that has not adapted to the changed environment. This proposition proves to be true in that the observed effect was the most expressed in the samples of the variant with a high dose of applied compost. Despite it, even by the end of the second growing season, the level of C_{mic} in the samples of the experimental variants remained above that of the control variant. The data of literature about the response of microbial community to the components of the sewage sludge or composts on the basis of the sediments are contradictory. Therefore, it was shown that the application of an organic substance as part of the pellets of sewage or composts on the basis of the pellets led to an increase in the microbial biomass of soils, especially in the first month after application (Fernandes et al., 2005). At the same time, the absence of a positive effect and even in some cases the inhibition of a microbial biomass was marked as well (Barajas-Aceves et al., 2005). Despite the absence of a decrease in the level of the biomass in our experiment, we compared the values of the content of metals in the samples of soils of our experiment (Table 1) with the concentrations reducing the level of a microbial biomass, which were presented in the literature (McGtath et. al., 1995; Giller et al., 1998, 2009; Barajas-Aceves et al., 2005). It was shown that, in the investigated samples of soils, the content of metals was below the values described in the literature, which is in accordance with the absence of a negative effect on the microbial biomass observed in our experiment.

The respiratory activity of microbial community of the luvisol of the control variant varied in the range from 0.02 to 0.26 mg CO_2–C/g*24 h (Fig. 3). It should be noted that despite the low content of organic substance in the soil used in the experiment, the level of the respiratory activity of the soil was comparable to the activity of richer soils, which contain an organic substance at a level of 1.5–2% (Bastida et al., 2008; Chodak et al., 2013).

The respiratory activity of the soil samples of the experimental plots, which were selected immediately after the compost application, was, as a whole, commensurable with the control variant (Fig. 3). In the samples of the experimental variants, the values of respiration were authentically higher by 66 and 124 days of research. The dose-dependent effect was noted. In the second vegetative season, the mineralizing activity of experimental samples was commensurable with that of the control variant, and the absolute values of activity were significantly lower than the activity in the first vegetative season.

Thus, the evaluation of the intensity of respiratory activity of soil during two vegetative seasons allowed us to reveal the stimulating effect of the compost application during the first vegetative season and the essential decrease in activity practically to the level of the control variant during following season.

According to the data of literature, the level of respiratory activity can both increase and decrease as a result of the compost applications. The authors of some publications noted the stimulating effect 1–2 years after the application of composts from the sewage sludge (Fernandes et al., 2005). The authors associated the stimulation with the favorable influence of the additional organic matter. At the same time, the increase in respiratory activity can be also related to the presence of toxic components, in particular metals, by means of an additional energy load on the soil microorganisms linked to the tolerance formation. In this case the applied substratum is consumed more for the respiration of microorganisms than for the formation of cells (Giller et al., 1998). According to the data of literature, this effect was observed in the case of such contents of Cd, Cu, Ni, and Zn in the applied pellet of sewage as 815, 182, 98, and 325 mg/kg, respectively, (Moreno et al. 1999; Khan and Scullion, 2000). At the same time, according to the data of other authors, the presence of metals in soil can induce a decrease in soil respiration. Such a decrease was revealed in the case of the presence of 25 and 50 mg/kg of Cd, 800 mg/kg of Zn, and 500 mg/kg of Pb in soil (Dar, 1994; Rost et al., 2001; Dar, 1997). The comparison of the data of the literature with the content of the metals in the samples of investigated soils shows that in this case the content of the metals was lower than the values at which essential damages of the energy metabolism of microbial cells were observed (McGtath et al., 1995).

Over two growing seasons, the values of metabolic quotient of the plots untreated with compost changed from 0.05 to 2.47 mg CO_2–C /g*24 h (Fig. 4). During both growing seasons the qCO_2 values of the soil samples of the Kl, Km, and Kh variants were essentially lower than the values of the samples of control variant (Fig. 4). This regularity is quite explainable and related to the high level of microbial biomass in these samples. It should be noted that the values of metabolic quotients during the second growing season were 1.2–3 times lower in comparison with those of the first growing season.

According to the literature data, an increase of metabolic quotient is used as a universal indicator of soil stress under the influence of metals (Brooks, 1995; Giller et al., 1998; Moreno et al., 1999; Jiang et al., 2003). Therefore, the absence of an essential increase in the values of metabolic quotient in our study can be evidence of the absence of negative influence on the microbial community of the soil.

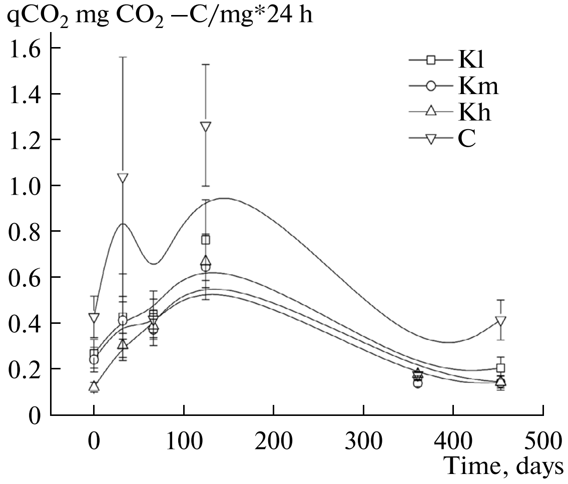

Fig. 4. Change in metabolic quotient over the time of the experiment.

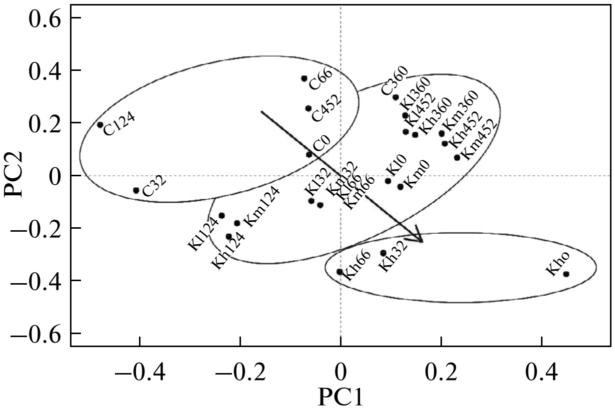

Fig. 5. Analysis of the data by the method of principal components. The letters in the captions to points indicate the variant of the treatment of soil; the numbers indicate the time of the selection of samples.

Thus, the data characterizing the microbial communities selected in various time intervals from plots containing different quantities of organic substance and metals were obtained. However, the totality of these results did not allow us to directly reveal the general regularity in the response of microbial communities. Therefore, at the final stage, the ordination analysis by the method of principal components was performed (Fig. 5). For this purpose, each of the analyzed soil samples was presented as a vector from three following numerical attributes: biomass, respiration, and metabolic quotients. Each of the objects was ciphered in the form of the name of an analyzed variant and the time of the sample selection. The data were standardized by the subtraction of an average and division on a standard deviation. The analysis allowed us to select

Table 3. Results of the variance analysis of the correlation of the indicators of microbial biomass, respiratory activity, metabolic quotient, and factors (C_{org} and metals)

Factor	Coefficient of canonical correlation	Explained variance of the totality of indicators, %	Fisher's statistic (F)	Level of significance
C_{org}	0.85	72.1	56.9	<0.001
Cd	0.61	37.8	13.4	0.001
Cr	0.80	64.2	39.5	<0.001
Cu	0.71	50.9	22.8	<0.001
Ni	0.67	45.4	18.3	<0.001
Pb	0.58	33.7	11.2	0.003
Zn	0.74	54.6	26.5	<0.001

Table 4. Results of the variance analysis (level of significance) of the indicators of the microbial community

Indicators of microbial community	Factors						
	C_{org}	Cd	Cr	Cu	Ni	Pb	Zn
Microbial community	<0.001	0.316	0.001	0.024	0.024	0.030	0.165
Respiratory activity	0.005	0.002	0.001	0.012	0.007	0.014	0.001
qCO_2	0.001	0.553	0.045	0.011	0.802	0.291	0.140
Totality of indicators	<0.001	0.021	<0.001	0.002	0.006	0.038	0.001

three groups consecutively located along a line reflecting the change in the C_{org} content. The arrangement of groups has a direction from the C0–C452 samples (with low content of organic substance) to the Kh0, Kh32, and Kh66 samples, as characterized by the maximum C_{org} content.

At the final stage, the variance of the data was analyzed (Table 3). To exclude the influence of the change of the indicators in time on the variability of the indicators, the dependence of the indicators on time was preliminarily removed by linear regression. The analysis of variance of canonical correlation of the set of indicators of biomass, respiration, and qCO_2 to the C_{org} content and concentrations of metals within the limits of linear model showed that the C_{org} factor explains 72.1% of the dispersion (the significance level was <0.001), whereas this value of all other factors was essentially lower, on average being 47.8%. Additionally, the multivariate and one-way analysis of variance was carried out. In Table 4, significance levels for the multivariate analysis of variance (MANOVA) and the one-way analysis of the variability of some indicators depending on the C_{org} content and the concentration of metals are presented. The data confirmed that influence of the content of organic substance was higher than the influence of metals.

CONCLUSIONS

In the course of the application of composts from the sewage sludge, the simultaneous inflow of organic substance and metals in soil leads to a change in the microbial communities evaluated according to the level of a microbial biomass, respiratory activity, and metabolic quotient. For two seasons after soil treatment, in the case of the C_{org} content reaching 2.1 ± 0.4% and mobile forms of Cd, Cr, Si, Ni, Pb, and Zn reaching 1.1 ± 0.03, 3.8 ± 0.8, 6.0 ± 1.2, 2.1 ± 0.5, 3.2 ± 0.7, and 12.3 ± 2.7 kg/mg in soil, respectively, an increase in the level of a microbial biomass in comparison with control soil, which contained C_{org} 0.7 ± 0.04% and mobile forms of Cd, Cr, Cu, Ni, Pb, and Zn of 0.2 ± 0.02, 1.5 ± 0.03, 2.0 ± 0.5, 1.0 ± 0.2, 1.2 ± 0.3, and 6.2 ± 1.5 mg/kg, respectively, was observed. The respiratory activity of soils of the experimental variants increased during the first season and decreased by the end of the second season to the level of the control variant. These effects are related to the removal of limitation in carbon that soil microorganisms traditionally undergo. The calculated metabolic quotient, whose level does not exceed the level of the control variant, indicates that in these conditions the microbial community is not under stress. According to the data of the principal component analysis, the content of organic substance in soil is a major factor determining the variability of microbial community.

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