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SOIL BIOLOGY

The Influence of Bacterial–Humus Preparations on the Biological Activity of Soils Polluted with Oil Products and Heavy Metals

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Abstract—The influence of bacterial–humus preparations based on *Gumigel* (*Agrosintez* Company) on the biological activity of soddy-podzolic soil polluted with $Pb(CH_3COO)_2$ and gasoline was studied in a model experiment. Some indicators of biological activity are shown to depend on soil pollution to different extents. The process of nitrogen fixation and the activity of dehydrogenase and phosphatase were mostly inhibited by $Pb(CH_3COO)_2$ and gasoline. Gasoline compared to $Pb(CH_3COO)_2$ inhibited the soil biological activity to a greater extent. The bacterial–humus preparations exerted a significant positive effect on the biological activity of the polluted soils manifested in the increase of the total number of bacteria and of the enzyme activity (1.5–5.0 times), in the intensification of nitrogen fixation and denitrification (3–8 times), as well as in the increase in the biomass of the plants grown (1.5–2.0 times). The application of bacterial suspensions of pure cultures or the microbial complex without the preparations of humic acids did not always give a positive effect.

Keywords: bacterial–humus preparations, biological activity of soils, soil remediation

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INTRODUCTION

The continuously increasing anthropogenic impact on the environment is accompanied by entering of toxic compounds to ecosystems. Among the priority pollutants are oil hydrocarbons and heavy metals. Being accumulated in the soil, they determine its toxicity for living organisms. Soils with a high content of pollutants become unsuitable for the normal growth of plants. The permanent increasing of the area of polluted lands makes the problem of their remediation topical. The use of bacterial preparations based on humic acids (bacterial–humus preparations) may be a prospective method for the restoration of soil fertility. The capability of humic acids to exhibit protective properties relative to soil microorganisms and plants is well known [3, 7, 10]. Humic acids can bind the toxicants into complexes unavailable for plants. They also improve the physicochemical properties of soils. Bacteria can decompose the organic pollutants (oil hydrocarbons, xenobiotics). The microbial biomass can sorb heavy metals [2, 6, 11]. Microorganisms synthesize the physiologically active substances stimulating the growth of plants [4]. Bacterial–humus preparations combine the properties of humic acids and bacterial cultures. In addition, humic acids can also support the viability of microorganisms in the preparation introduced into the soil [5, 12].

The objective of this work is to study the effect of bacterial–humus preparations based on *Gumigel* (*Agrosintez* Company) on the biological activity of soils polluted with oil products and heavy metals.

OBJECTS AND METHODS

The studies were conducted with two bacterial–humus preparations representing a solution of potassium humate isolated from brown coal (Gumigel, Agrosintez Company) enriched with the following: (1) a mixture of pure *Rhodococcus* and *Pseudomonas* bacterial cultures (further HA + BC), and (2) a complex of microorganisms (HA + SC) isolated from a mucky–gley soil (Training and Experimental Soil–Ecological Center Chashnikovo, Moscow State University) and containing bacteria of the *Bacillus*, *Arthrobacter*, *Rhodococcus*, *Pseudomonas*, *Cytophaga*, *Myxococcus*, and *Aquaspirillum* genera. *Gumigel* is a dark brown colloidal gel of potassium humate with pH 7.0, a density of 1.1 g/cm^3, and a mass humate fraction of 5%. The titer of bacteria after the introduction of humate into the initial preparation was 10^9 cell/mL. Model tests were performed in vessels with a volume of 0.3 L to study the effect of the bacterial–humus preparations on the biological activity of the soils. For the experiments, soddy-podzolic soils (Tomilino Forest Park, Moscow oblast) were used. Gasoline AI-95 at a concentration of 4% and $Pb(CH_3COO)_2$ at a concentration of 5MPC were applied as pollutants. The bacterial–humus preparations (at the volume of 40 mL/kg) were added to the soils. In order to determine the contribution of the humic and bacterial components to the biological activ-

ity of the soils, experiments with the application of bacterial *Rhodococcus* and *Pseudomonas* suspensions, suspensions of the microbial complex from the mucky–gley soil (the bacterial titer is 10^9 cell/ml), and a sterile preparation of humic acid (Gumigel) were performed. The soil without gasoline and $Pb(CH_3COO)_2$ was used as a control. A total of 18 variants were tested in the model experiment: (1) C—the control soil; (2) HA—the soil with the application of sterile humic acid; (3) BS—the soil with the application of *Rhodococcus* and *Pseudomonas* bacterial suspensions; (4) MC—the soil with the application of the microbial complex of the mucky–gley soil; (5) HA + BS—the soil with the application of humic acids enriched with *Rhodococcus* and *Pseudomonas* bacteria; (6) HA + MC—the soil with the application of humic acids enriched with the microbial complex of the mucky–gley soil; (7) Pb—the soil contaminated with $Pb(CH_3COO)_2$; (8) Pb + HA—the soil contaminated with $Pb(CH_3COO)_2$ and sterile humic acid; (9) Pb + BS—the soil contaminated with $Pb(CH_3COO)_2$ and the application of *Rhodococcus* and *Pseudomonas* bacterial suspensions; (10) Pb + MC—the soil contaminated with $Pb(CH_3COO)_2$ and the application of the microbial complex of the mucky–gley soil; (11) Pb + HA + BS—the soil contaminated with $Pb(CH_3COO)_2$ and the application of the humic acid preparation enriched with *Rhodococcus* and *Pseudomonas* bacteria; (12) Pb + HA + MC—the soil contaminated with $Pb(CH_3COO)_2$ and the application of the humic the humic acid preparation enriched with the microbial complex of the mucky–gley soil; (13) G—the soil contaminated with gasoline; (14) G + HA—the soil contaminated with gasoline with the application of sterile humic acid; (15) G + BS—the soil contaminated with gasoline with the application of *Rhodococcus* and *Pseudomonas* bacterial suspensions; (16) G + MC—the soil contaminated with gasoline and the application of the microbial complex of the mucky–gley soil; (17) G + HA + BS—the soil contaminated with gasoline and the application of the humic acid preparation enriched with *Rhodococcus* and *Pseudomonas* bacteria; and (18) G + HA + MC—the soil contaminated with gasoline and the application of humic acid preparation enriched with the complex of microorganisms from the mucky–gley soil.

The influence of the bacterial–humus preparations on the biological activity of the soils was assessed according to the changes in the total bacterial number, the respiration intensity of the soil microorganisms, the nitrogen fixation, the denitrification and activity of catalase, the dehydrogenase, the urease, and the phosphatase. The structure of the saprotrophic bacterial complex was also studied.

The total number of bacteria was determined by luminescence microscopy using painting with acridine orange [9]. The respiration intensity, the activity of nitrogen fixation, and the denitrification by the soil microorganisms were analyzed using the method of gas chromatography [9]. The catalase activity was determined by the Galstyan gasometric method [9]. For the analysis of the dehydrogenase activity, 2,3,5-triphenyl tetrazolium chloride, which is reduced to a red compound, triphenylformazan, was applied. The amount of the triphenylformazan formed was determined using a colorimeter [9]. The urease activity was estimated using the colorimetric measurement of the colored solution that is formed as a result of the reaction with Nessler's reagent due to ammonia isolated upon the urea decomposition [9]. For the colorimetric analysis of the phosphatase activity, the amount of phenolphthalein, which is formed by the decomposition of phenolphthalein phosphate, was determined [1].

For studying the effect of the bacterial–humus preparations on the structure of the bacterial community, the method of plating on an agar glucose–peptone–yeast medium was applied [8]. The plating was traditionally performed from 1 : 100 and 1 : 10000 dilutions (five replicates) after the preliminary treatment on a UZDN-1 ultrasound dispersant (22 kHz, 0.44 A, 2 min). In order to inhibit the development of fungi, 50 mg of nystatin was added for 0.5 L of the medium.

The effect of the preparations on plants was determined according to the biomass of *Lepidium sativum* in a pot experiment. Seeds of the salad (50 seeds) were sown in vegetative pots with soddy-podzolic soil (300 g). The duration of the model experiment was 3 months.

RESULTS AND DISCUSSION

The studies conducted showed that the pollution of the soils with gasoline and $Pb(CH_3COO)_2$ significantly affected all the indices of the soil biological activity except for the respiration of the soil microorganisms.

Changes in the total number of bacteria. The 4% gasoline pollution of the soil has led to a reduction in the total bacterial number by 3 times (Fig. 1). The introduction of the bacterial–humus preparations to the polluted soil promoted the increase in the number of soil bacteria by 70%. A similar pattern was observed upon the $Pb(CH_3COO)_2$ pollution of the soils.

Estimation of the soil enzyme activity. Enzymes of oxidoreductases (catalase and dehydrogenase) and hydrolases (urease and phosphatase) are considered in this work.

The control soil was enriched with catalase to a medium extent according to the Zvyagintsev scale [9] (Fig. 2a). The gasoline pollution inhibited the catalase activity to the greatest extent. In this case, compared to the control, the catalase activity was 3 times lower. The soil polluted with gasoline was referred to the soils poor in catalase. The application of bacterial–humus preparations increased the catalase activity by 60–80%. The $Pb(CH_3COO)_2$ pollution decreased the catalase activity to a lesser degree than in the case with the gasoline pollution.

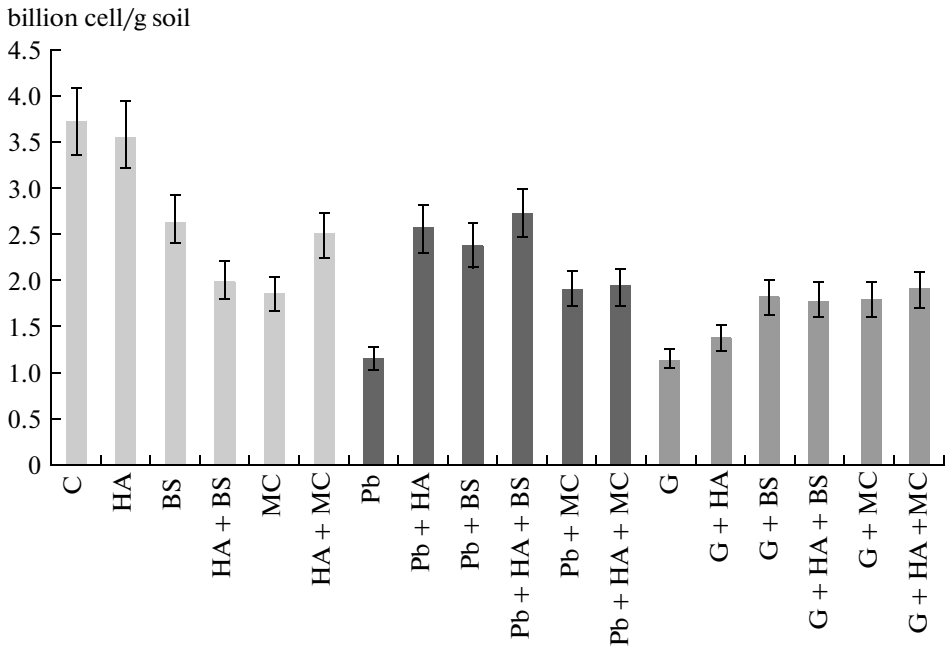

Fig. 1. Total number of bacteria.

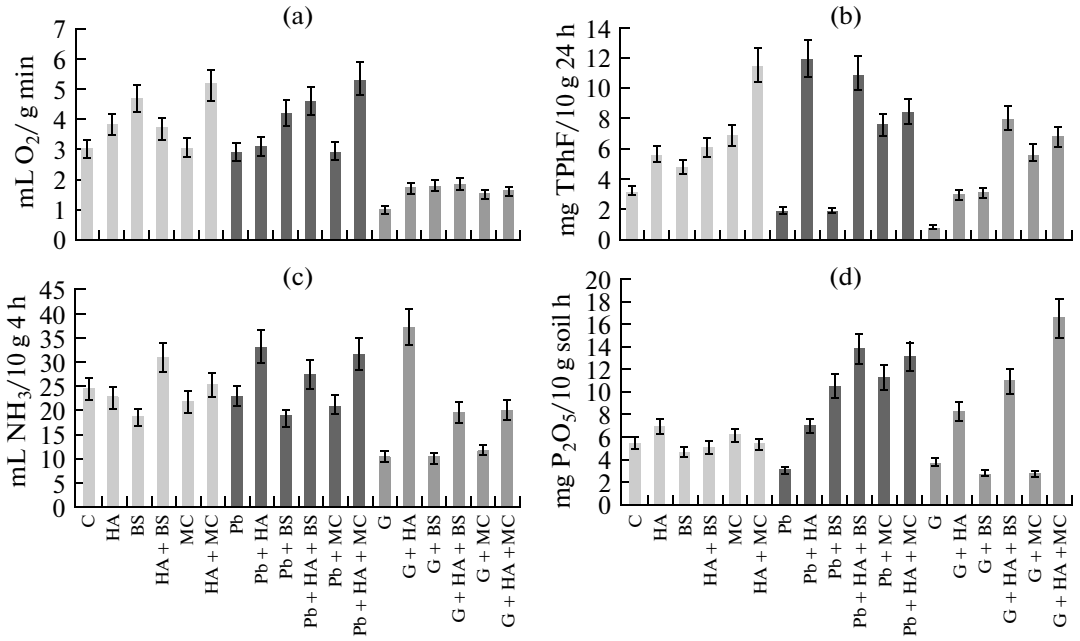

Fig. 2. Changes in the activity of the soil enzymes: catalase (a), dehydrogenase (b), urease (c), and phosphatase (d).

By the dehydrogenase activity, the control soil was referred to those with the medium content of this enzyme (Fig. 2b). The introduction of the bacterial–humus preparations increased the dehydrogenase activity of the soil by 1.5–2.0 times. The soil with the bacterial–humus preparations enriched with the microorganisms from the mucky–gley soil was characterized as a rich one in terms of the dehydrogenase activity. The gasoline pollution reduced the dehydrogenase activity by more than 3 times; according to the content of this enzyme, this soil was referred to very poor ones. The use of the bacterial–humus preparations in the gasoline-polluted soil promoted the increase in the dehydrogenase activity by 3–9 times; the amount of this enzyme in the soil was medium. $Pb(CH_3COO)_2$ inhibited the dehydrogenase activity

to a greater extent than the catalase activity. As compared to the control soil, the dehydrogenase activity decreased almost by 2 times; it was poor in terms of the enrichment with this enzyme. The application of the bacterial–humus preparations in the $Pb(CH_3COO)_2$ polluted soil 5–6 times increased the dehydrogenase activity. Thus, the dehydrogenase activity, compared to that of catalase, is a more sensitive indicator of the soil biological activity.

The control soil was medium in terms of the urease content (Fig. 2c). The gasoline pollution decreased the urease activity by 2.5 times. The application of the bacterial–humus preparations increased the urease activity by 2–3 times, while the introduction of simple bacterial suspensions to the contaminated soil did not positively affect them. The character of the influence of $Pb(CH_3COO)_2$ on the urease activity was similar to that of catalase. The enzyme activity was 10% reduced. The application of bacterial suspensions, as in the case with gasoline pollution, did not have any significant influence.

In terms of the phosphatase content, the control soil was rich (Fig. 2d). The gasoline pollution decreased the phosphatase activity by 2 times up to the medium enrichment of the soil with this enzyme. The use of the bacterial–humus preparations increased the phosphatase activity by 2.5–5.0 times. The application of the bacterial suspensions, as the use of urease, did not have any effect. $Pb(CH_3COO)_2$ inhibited the phosphatase activity by 2 times, as well as gasoline did. However, in this case, both the bacterial–humus preparations and bacterial suspensions did not positively affect the enzyme activity.

Thus, the dehydrogenase and phosphatase activities turned out to be the most sensitive characteristics. Gasoline most significantly inhibited the activity of all the enzymes in the soils. $Pb(CH_3COO)_2$ little influenced the catalase and urease activities but noticeably affected the dehydrogenase and phosphatase activities. The application of the bacterial–humus preparations exerted the most positive influence on the enzyme activity. The use only of bacterial cultures did not positively affect the urease and phosphatase activities in the gasoline-contaminated soils and the urease activity in the $Pb(CH_3COO)_2$ polluted soil.

Changes in nitrogen fixation and denitrification. The contamination of the soils decreased the activity of nitrogen-fixing microorganisms to a greater extent (Fig. 3a). Upon $Pb(CH_3COO)_2$ pollution, the nitrogen fixation was lowered by more than an order of magnitude, while upon the gasoline pollution, by almost 2 orders. The application of the bacterial–humus preparations in the gasoline-polluted soil intensified the nitrogen fixation by 5 times. The positive effect of the bacterial–humus preparations was 20% higher than that in case of the application of the bacterial suspensions. With the use of these compounds in the $Pb(CH_3COO)_2$ polluted soil, the nitrogen fixation increased by 3–8 times. The positive effect of applying

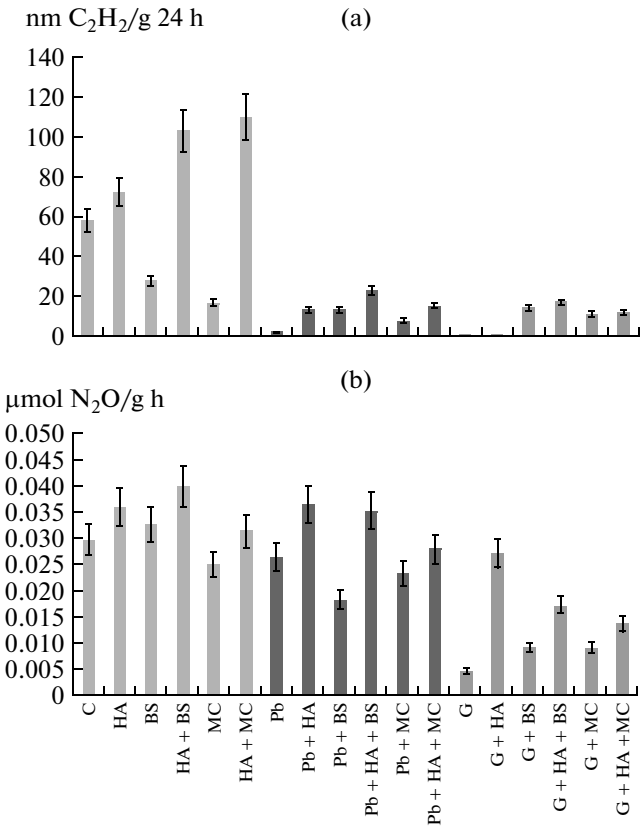

Fig. 3. Changes in the activity of nitrogen fixation (a) and denitrification (b).

the bacterial–humus preparations was twice higher than that of the bacterial suspensions.

In the gasoline-contaminated soil, the denitrification became lower by 6 times (Fig. 3b); the use of the bacterial–humus preparations increased it by 3–6 times. The positive effect of the bacterial–humus preparations exceeded that from the bacterial suspensions by 50–90%. A less inhibiting effect was observed in the $Pb(CH_3COO)_2$ polluted soil. The denitrification was 10% reduced. The use of the bacterial–humus preparations intensified the denitrification by 1.5 times.

Changes in the respiration intensity of the soil microorganisms. The respiration of the soil microorganisms changed to the least extent as compared to the changes in the other indices of the soil biological activity (Fig. 4). In the gasoline-polluted soils, the CO_2 emission increased by 1.5 times due to the use of an additional carbon source. The low difference in the CO_2 emissions between the test variants can be also explained by the considerable contribution of fungi to the soil respiration. Fungi are known to be more resistant to stress; therefore, gasoline little affected their metabolic activity. This is also true for $Pb(CH_3COO)_2$.

Changes in the structure of the saprotrophic bacterial complex. The relative abundance of bacteria from

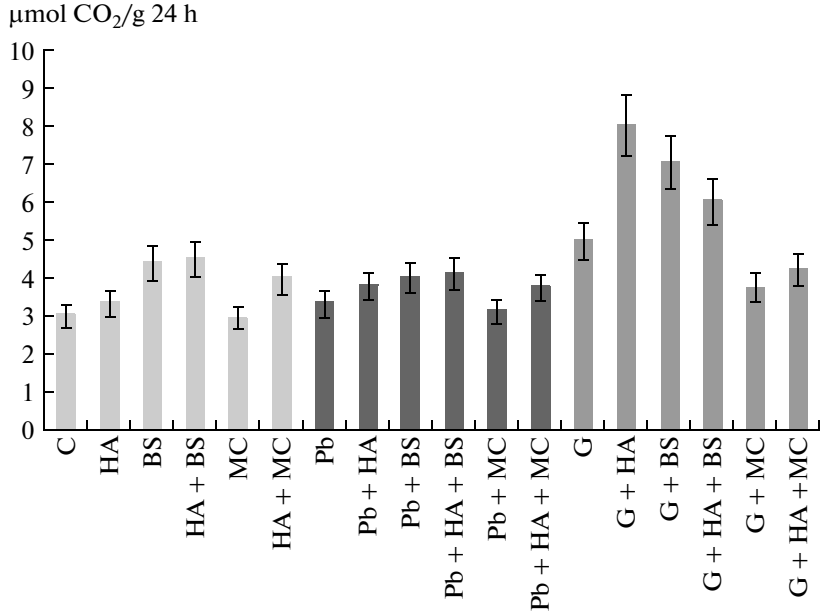

Fig. 4. Respiration of soil microorganisms.

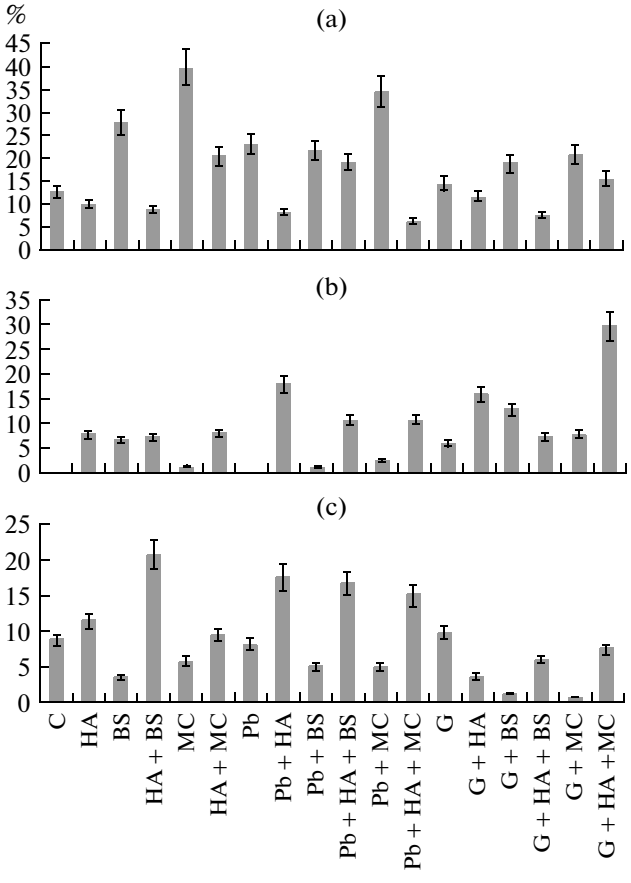

Fig. 5. Changes in the share of *Bacillus* (a), *Pseudomonas* (b), and *Rhodococcus* (c) bacteria in the structure of the saprotroph bacterial complex.

the genera studied was used for the characterization of the saprotrophic bacterial complex structure according to the following gradations: dominants—more than 30% of the total number of bacteria counted on the applied medium; subdominants—20–30%; the group of medium abundance—10–20%; and the minor components—less than 10%.

The analysis of the structure of the saprotrophic bacterial complex showed that, in most cases, the application of the bacterial–humus preparations promoted an increase in their diversity, which is estimated by the number of genera determined (Table 1). The soil pollution caused an increase in the share of *Bacillus* bacteria capable of enduring unfavorable conditions by forming spores. As the bacterial–humus preparations were applied, the share of *Bacillus* bacteria significantly decreased (Fig. 5a). Bacteria of the *Rhodococcus* and *Pseudomonas* genera that often are present in the preparations used for the remediation of oil-polluted soils are of a special interest. The studies showed that $Pb(CH_3COO)_2$ in the polluted soils inhibited the development of *Pseudomonas* bacteria to a great extent (Fig. 5c). During the experiment, it was not possible to isolate representatives of this genus from the $Pb(CH_3COO)_2$ polluted soil without the application of any preparations. The introduction of bacterial suspensions to the soil increased the share of *Pseudomonas* bacteria up to 0.9 and 2.0% in the pure *Rhodococcus* and *Pseudomonas* cultures and in the soil microbial complex, respectively. In this case, *Pseudomonas* bacteria are in the group of minor components. The application of the bacterial–humus preparations has caused a significant increase (more than 10%) in the share of

Table 1. Changes in the structure of the saprotrophic bacterial complex

Variant	Dominants	Subdominants	Average abundance	Minor components
C	*Arthrobacter*		*Bacillus*	*Myxococcus, Rhodococcus, Streptomyces, Polyangium, Cytophaga,Vibrio, Erwinia, Cellulomonas*
HA		*Arthrobacter*	*Rhodococcus, Myxococcus, Streptomyces*	*Cytophaga,Vibrio, Proteus, Pseudomonas, Bacillus, Xantomonas, Erwinia*
BS	*Arthrobacter*	*Bacillus*	*Myxococcus*	*Rhodococcus, Spirillum, Pseudomonas, Streptomyces, Cytophaga*
HA + BS		*Rhodococcus*	*Arthrobacter, Cytophaga*	*Bacillus, Myxococcus, Pseudomonas, Streptomyces*
MC	*Bacillus*	*Arthrobacter*	*Streptomyces*	*Rhodococcus, Vibrio, Myxococcus, Erwinia, Cytophaga, Polyangium, Pseudomonas*
HA + MC		*Bacillus*, *Arthrobacter*	*Myxococcus*	*Rhodococcus, Vibrio, Pseudomonas, Streptomyces, Cytophaga, Erwinia, Cellulomonas*
Pb	*Arthrobacter*	*Bacillus*		*Rhodococcus, Vibrio, Myxococcus, Proteus, Streptomyces, Cytophaga, Cellulomonas, Enterobacter*
Pb + HA			*Arthrobacter, Rhodococcus, Pseudomonas, Vibrio*	*Bacillus, Myxococcus, Streptomyces, Erwinia, Cytophaga, Polyangium*
Pb + BS		*Bacillus*, *Arthrobacter*	*Myxococcus, Streptomyces,*	*Rhodococcus*, *Proteus*, *Pseudomonas*, *Cytophaga*, *Polyangium*, *Cellulomonas*
Pb + HA + BS	*Arthrobacter*		*Rhodococcus, Pseudomonas, Bacillus*	*Myxococcus, Cytophaga, Streptomyces, Vibrio, Polyangium, Cellulomonas*
Pb + MC	*Bacillus*, *Arthrobacter*		*Streptomyces*	*Rhodococcus, Vibrio, Myxococcus, Spirillum, Pseudomonas, Cellulomonas*
Pb + HA + MC	*Arthrobacter*		*Rhodococcus,Myxococcus, Pseudomonas, Streptomyces*	*Bacillus, Cytophaga, Erwinia, Polyangium, Vibrio, Cellulomonas, Enterobacter, Serratia*
G	*Vibrio*		*Bacillus, Myxococcus*	*Arthrobacter, Pseudomonas, Streptomyces, Erwinia, Rhodococcus, Cellulomonas*
G + HA	*Arthrobacter*	*Myxococcus*	*Bacillus, Pseudomonas*	*Rhodococcus, Vibrio, Streptomyces, Cytophaga, Alcaligenes*
G + BS	*Myxococcus*		*Bacillus, Aeromonas, Pseudomonas*	*Arthrobacter, Vibrio, Rhodococcus, Proteus, Cytophaga*
G + HA + BS	*Arthrobacter*	*Myxococcus*		*Bacillus,Rhodococcus, Pseudomonas, Vibrio, Streptomyces, Cytophaga, Enterobacter, Aeromonas*
G + MC	*Myxococcus*	*Bacillus*	*Arthrobacter, Vibrio*	*Rhodococcus, Pseudomonas, Spirillum*
G + HA + MC		*Pseudomonas*	*Bacillus, Arthrobacter*	*Myxococcus, Cytophaga, Enterobacter, Proteus, Alcaligenes, Aeromonas, Rhodococcus, Vibrio*

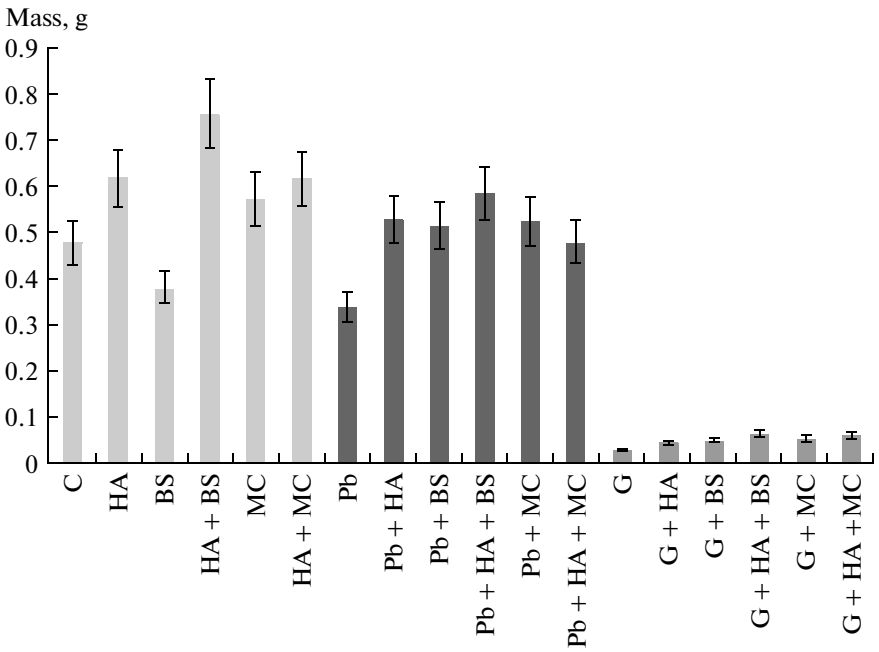

Fig. 6. Changes in the biomass of cress salad.

representatives of this group. Thus, bacteria of this group could be referred to the group of medium abundance. The gasoline contamination exerted a less adverse effect on the development of *Pseudomonas* bacteria as compared to the influence of $Pb(CH_3COO)_2$, since gasoline components can be used as a substrate. The use of the bacterial–humus preparations also promoted the increase in the proportion of *Pseudomonas* bacteria.

The soil pollution less adversely affected the development of *Rhodococcus* bacteria than that of the *Pseudomonas* representatives. However, in this case, the positive influence of the bacterial–humus preparations was also visible (Fig. 5c). The effect from using the bacterial–humus preparations was 3–5 times higher than the positive action from the application of the bacterial suspensions.

Cress-salad biomass. The 4% gasoline contamination of the soils reduced the biomass of the cress-salad by an order of magnitude. The use of the bacterial–humus preparations increased its biomass by 1.5–2.0 times. Their effect was 10–20% higher than that of the bacterial suspensions (Fig. 6). The $Pb(CH_3COO)_2$ pollution reduced the biomass by 25%. The application of the bacterial–humus preparations favored the increase in the plant biomass by 40–50%; a faster transition from the stage of vegetation to the flowering stage was observed.

Statistical analysis of the results of the model experiment. The variance analysis of the results was performed to reveal what factors (type of pollution, application of humic acids, introduction and type of microbial complex) affect some characteristics of the soil biological activity. For the classification of the test variants, cluster analysis was used.

The variance analysis has revealed the following (Table 2). The most sensitive characteristics of the soil biological activity turned out to be the total number of bacteria, the enzyme activity, and the denitrification. All the factors considered affected these characteristics.

The nitrogen fixation was influenced by the soil pollution ($p = 0.00$) and the humic acids applied ($p = 0.0497$). The introduction of any bacteria did not affect the nitrogen fixation.

Only one factor, the soil pollution, influenced the respiration activity of the microorganisms ($p = 0.0002$). This fact agrees with the above described changes in the CO_2 emission.

Using the cluster analysis of the Word method, the test variants were divided into several classes. This analysis also enables one to divide the variants into conventional groups (Fig. 7):

unpolluted soil (contains 3 objects: C, HA, HA + BS);

restored soil (contains 10 objects: BS, MC, HA + MC, Pb + HA, Pb + HA + BS, Pb + MC, Pb + HA + MC, G + HA + BS, G + MC, G + HA + MC);

polluted soil (contains 5 objects: Pb, Pb + BS, G, G + HA, G + BS).

Thus, the cluster analysis has confirmed the efficiency of using bacterial–humus preparations for the restoration of the biological activity of contaminated soils.

Table 2. Variance analysis of the influence of the soil pollution and remediation method on the characteristics of the soil biological activity

Factor	Sum of squares	Number of degrees of freedom	Average square	*F*-criterion	Significance level
		Number			
Pollution	10.6403	2	5.3201	103.947	0.0000
HA	0.6241	1	0.6241	12.193	0.0013
Bacteria	0.7893	2	0.3947	7.711	0.0016
Pollution + HA	1.0595	2	0.5297	10.350	0.0003
Pollution + bacteria	10.2263	4	2.5566	49.951	0.0000
HA + bacteria	0.8217	2	0.4109	8.028	0.0013
Pollution + HA + bacteria	2.0031	4	0.5008	9.784	0.0000
		Catalase			
Pollution	63.5025	2	31.7513	48.3829	0.0000
HA	5.5104	1	5.5104	8.3968	0.0064
Bacteria	7.8325	2	3.9163	5.9676	0.0058
Pollution + HA	1.1558	2	0.5779	0.8806	0.4233
Pollution + bacteria	1.8200	4	0.4550	0.6933	0.6015
HA + bacteria	6.6658	2	3.3329	5.0787	0.0114
Pollution + HA + bacteria	5.4367	4	1.3592	2.0711	0.1050
		Dehydrogenase			
Pollution	1437.37	2	718.69	19.465	0.0000
HA	4971.30	1	4971.30	134.644	0.0000
Bacteria	2361.83	2	1180.91	31.984	0.0000
Pollution + HA	1023.52	2	511.76	13.861	0.0000
Pollution + bacteria	988.49	4	247.12	6.693	0.0004
HA + bacteria	533.01	2	266.50	7.218	0.0023
		Urease			
Pollution	824.89	2	412.45	47.810	0.0000
HA	79.37	1	79.37	9.200	0.0045
Bacteria	179.47	2	89.74	10.402	0.0003
Pollution + HA	83.93	2	41.96	4.864	0.0135
Pollution + bacteria	553.05	4	138.26	16.027	0.0000
HA + bacteria	3.60	2	1.80	0.209	0.8125
Pollution + HA + bacteria	164.69	4	41.17	4.773	0.0034
		Phosphatase			
Pollution	22.8776	2	11.4388	8.4696	0.0010
HA	106.6049	1	106.6049	78.9331	0.0000
Bacteria	63.5260	2	31.7630	23.5182	0.0000
Pollution + HA	40.5947	2	20.2974	15.0287	0.0000
Pollution + bacteria	19.5796	4	4.8949	3.6243	0.0140
HA + bacteria	28.5728	2	14.2864	10.5780	0.0002
Pollution + HA + bacteria	22.3271	4	5.5818	4.1329	0.0074
		Denitrification			
Pollution	0.003268	2	0.001634	128.193	0.0000
HA	0.001668	1	0.001668	130.829	0.0000
Bacteria	0.000230	2	0.000115	9.007	0.0007
Pollution + HA	0.000161	2	0.000080	6.308	0.0045
Pollution + bacteria	0.000426	4	0.000106	8.353	0.0001
HA + bacteria	0.000253	2	0.000127	9.938	0.0004
Pollution + HA + bacteria	0.000580	4	0.000145	11.375	0.0000
		Nitrogen fixation			
Pollution	18419.98	2	9209.99	14.89111	0.0000
HA	2550.92	1	2550.92	4.12445	0.0497
Bacteria	3155.87	2	1577.93	2.55127	0.0920
Pollution + HA	2537.79	2	1268.89	2.05160	0.1433
Pollution + bacteria	6586.58	4	1646.64	2.66237	0.0481
HA + bacteria	2312.02	2	1156.01	1.86909	0.1689
Pollution + HA + bacteria	2238.28	4	559.57	0.90474	0.4715

Table 2. (Contd.)

Factor	Sum of squares	Number of degrees of freedom	Average square	*F*-criterion	Significance level
Respiration					
Pollution	28.602	2	14.301	11.231	0.0002
HA	1.605	1	1.605	1.261	0.2689
Bacteria	1.934	2	0.967	0.759	0.4753
Pollution + HA	1.244	2	0.622	0.488	0.6176
Pollution + bacteria	6.928	4	1.732	1.360	0.2670
HA + bacteria	7.112	2	3.556	2.792	0.0746
Pollution + HA + bacteria	1.866	4	0.467	0.366	0.8309

CONCLUSIONS

The soil pollution affected to different extents some characteristics of the biological activity of the soddy-podzolic soil investigated. In the contaminated soils, the nitrogen fixation and dehydrogenase and phosphatase activities were mostly inhibited. The respiration intensity by the soil microorganisms turned out to be the most resistant to soil pollution. The type of pollution also influenced the soil biological activity. Gasoline inhibited all the characteristics of the biological activity, except for the respiration; $Pb(CH_3COO)_2$ reduced the catalase and urease activities and denitrification to a lesser extent. The dehydrogenase and phosphatase activities and nitrogen fixation were strongly inhibited by $Pb(CH_3COO)_2$. In all the test variants, the application of the bacterial–humus preparations had the highest positive effect on the biological activity. The introduction of bacterial suspensions of pure cultures and the microbial complex without humic acids to the polluted soil sometimes had no

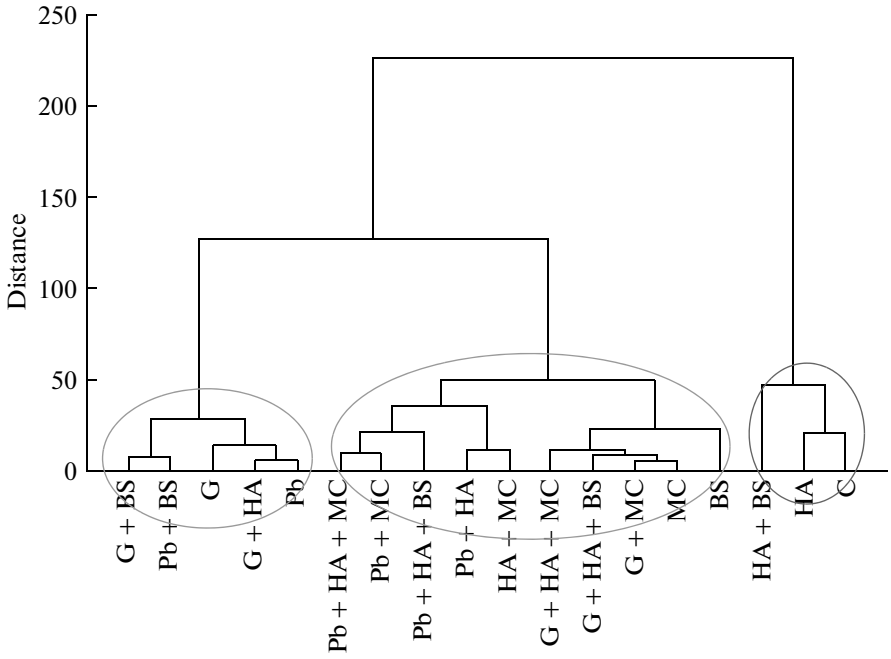

Fig. 7. Results of the cluster analysis.

effect. The biomass of the plants grown also depended on the type of pollution and the method of soil remediation. The gasoline contamination ten times lowered the plant biomass, while $Pb(CH_3COO)_2$ inhibited the growth of plants to a lesser extent. The bacterial–humus preparations stronger increased the plant biomass (by 10–20%) than the introduction only of the bacterial suspensions. In addition, humic acids enriched with the complex of microorganisms of the mucky–gley soil accelerated the transition of plants from the vegetation stage to the flowering stage.

One can conclude that the use of bacterial–humus preparations is highly efficient for the remediation of soils contaminated with heavy metals and oil products.

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