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DEGRADATION, REHABILITATION, AND CONSERVATION OF SOILS

Phytotoxicity of Heavy Metals in Contaminated Podzolic Soils of Different Fertility Levels

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Abstract—We have compared the impact of heavy metals (HMs: Cu 660 + Zn 1100 + Pb 650 mg/kg) on agrosoddy-podzolic soils (Albic Retisols (Loamic, Aric, Cutanic, Ochric)) of two arable fields (Chashnikovo, Moscow oblast) with different contents of organic carbon (C_{org} 3.86 and 1.30%) and different fertility levels using indicators of acute and chronic phytotoxicity. At the same level of polymetallic contamination, the responses of test plants to the presence of high concentrations of HMs and potential remediants (lignohumate and biochar) in soils of the same type with different C_{org} contents noticeably differ with respect to plant growth parameters and metal accumulation in the phytomass. The HMs contamination of low-fertile soil leads to the complete death of plants in the pot experiment, while plants on highly fertile soil continued to develop until flowering with only slight deviations from the control. Experimental data on the contents of total and water-soluble forms of HMs and nutrients in the studied soils are presented. The relationships between the chemical composition of soils and the results of phytotests have been found using the principal component analysis. It is shown that the threefold difference in the content of C_{org} between the soils of the same texture and acidity (pH) result in significantly different response of test plants to the same concentration of HMs. The necessity of correcting the standards for the permissible concentration of HMs in soils with due account for the C_{org} content in addition to pH and texture is discussed.

Keywords: environmental assessment of soils, bioassay, organic carbon, polymetallic pollution, rationing, lignohumate, biochar, bioavailability, heavy metals, copper, zinc, lead

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INTRODUCTION

Plant productivity and phytomass quality are the most important indicators of soil fertility. In this context, soil phytotoxicity is an essential indicator to the environmental assessment of the state of soils in agroecosystems [12, 21, 32, 38].

Toxic substances affect soil properties and thus may slow down the growth of plants; change their morphological properties; cause twisting, asymmetry, and shedding of leaves; and decrease enzymatic activity, which results in smaller productivity. One of the main mechanisms of the effect of toxicants is related to the formation of reactive oxygen compounds in plant cells: their high concentrations may completely suppress the antioxidant system and cause the oxidative stress and death of cells and the plant [5, 25, 30, 31, 34, 36].

Phytotesting in the express laboratory bioassay (germination of seeds in contact with water extract from soil or with soil in a wet chamber) during a short period (four or five days) provides data on acute phytotoxicity[1] [9, 21]. Chronic phytotoxicity is determined in a longer (from three to six weeks) pot experiment (ISO 22030:2005, Soil quality—Biological methods—Chronic toxicity in higher plants) [12]. These two methods are rarely used simultaneously, but it is important to understand the correlation between their results and to specify the test parameters of short-term experiments, which most adequately characterize the state of plants at late growing stages in order to predict the conditions for the entire growing season.

Soil contamination by heavy metals (**HMs**) and metalloids has long been an important environmental problem [2, 5, 7, 10, 13, 35, 36, 38]. Lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni) are the most common elements of this not clearly defined group [24].

[1] ISO 18763:2016. Soil Quality. Determination of the Toxic Effects of Pollutants on Germination and Early Growth of Higher Plants.

Organic and inorganic fertilizers, ameliorants, and irrigation water affect the accumulation and changes in the mobility of heavy metals in soils [6, 14, 18]. In turn, metal absorption by soils affects pH [1, 2, 5, 35], biological activity and structure of soil microbial communities [15, 23, 28–30, 35], and humus content and composition of humus [20].

Carbon-containing products, such as humic-based fertilizers of various origins [14, 16, 22, 27, 28, 37] and products of pyrolysis of wood and other organic wastes—(biochar) [14, 18, 19, 28]—are widely used to mitigate the toxic effect of different types of pollutants on soil.

Numerous studies, methodological recommendations, and guidelines are devoted to the chemical contamination of soils as a factor of environmental risk and reflect different methodological approaches to the assessment of the ecological status of soils[2]. Special attention is paid to the distribution of HMs in soils of agroecosystems [1, 6, 24, 38]. Widely applied data on the concentrations of chemicals cannot adequately characterize the state of biotopes [3, 8, 32]. The ranking of the environmental quality based on the content of chemical elements is insufficient because of different reaction of living systems to pollution [6, 15, 16]. It depends on many soil parameters, including soil acidity, texture, and the contents of nutrients. Nevertheless, existing standards on the permissible concentrations of pollutants (maximum permissible concentration (MPC) and tentative permissible concentration (TPC)) in soils play an important role in the system of ecological regulation, because they allow comparative assessments and can be used as more or less universal criteria, which is reflected in state hygienic standards (GN (Hygienic Standard) 2.1.7.2041-06, GN 2.1.7.2511-09) [2, 3, 6, 10, 11].

It is obviously important to identify the informative value of biotic indicators for assessing permissible impact of HMs on living systems for the soils with different organic carbon contents.

The aim of this work is to identify specific features of the development of higher plants in samples of agrosoddy-podzolic soils (Albic Retisols (Loamic, Aric)) with different fertility levels subjected to the same polymetallic contamination. The responses of test plants to the presence of high HM concentrations and potential remediants (biochar and lignohumate) in soils with different organic carbon contents were evaluated from data on plant growth parameters and the accumulation of metals in the phytomass.

OBJECTS AND METHODS

Soil. We analyzed samples of cultivated soddy-podzolic soil (Albic Retisols (Loamic, Aric, Cutanic, Ochric))[3] taken in two remote fields of Moscow oblast (Solnechnogorskii district, the Chashnikovo Training and Experimental Soil Ecology Center). Soil **S1** of the first field (56°02′01 N, 37°10′04 E) was attributed to well-cultivated (highly fertile) soils with a low acidity (pH_{KCl} 6.39 ± 0.05) and a high organic carbon content (C_{org} = 3.86%). Poorly cultivated (low-fertile) soil **S2** of the second field (56°01′41 N, 37°11′04 E) had pH_{KCl} 5.84 ± 0.05 and C_{org} = 1.30%. Soil samples were taken on test plots (40 m^2) of each field from the upper 20-cm layer using the rectangular sampling pattern in early May 2019. Soil samples taken on each field were averaged by mixing and transported to a laboratory. The sample weight was about 25 kg, and the initial water content was 35–40%. In the laboratory, the soil was air dried, purified from coarse plant fragments, passed through a 5-mm sieve, moistened to 55–60% of the field water capacity, and left for preincubation (five days at 22°C).

Heavy metals were applied to the soil samples as water solutions (10 mL/kg) of copper ($CuSO_4$), zinc ($ZnSO_4$), and lead ($PbCl_2$) to achieve the concentration of Cu, Zn, and Pb equal to 660, 1100, and 650 mg/kg soil, respectively, which amounted to five tentative permissible concentrations (TPC) of each metal (GN 2.1.7.2511-09).

Carbon-containing remediants (lignohumate and biochar) were added to the experimental samples of uncontaminated and metal-spiked soils separately and in combination.

Biochar (a pyrolysis product of birch wood, fraction 2–8 mm, produced by Metakom LLC, Russia) was added at the rate of 5% of the soil sample weight. It contained C (88.2%); N, H, and S (0.44, 0.82, and 0.19%, respectively); and ash (2.8%). Its pH_{CaCl_2} was 8.9. The content of Cu, Zn, and Pb cations in the biochar did not exceed 0.02% of its mass.

Potassium lignohumate was obtained by artificial humification of lignosulfonate (manufactured by the RET Research and Production Association, Russia). The ash content in the lignohumate was 40%; it contained 37.3, 0.5, 3.72, 4.84, and 9.0% of C, N, H, S, and K, respectively; and pH_{CaCl_2} was 9.0 (1% solution). Humic acids comprised 58% of organic matter. Lignohumate was applied to the soil as 10% water solution at the rate of 0.25% of the soil mass.

Design of the experiment. Samples of fertile (S1) and low-fertile (S2) soils were divided into two equal parts, and a mixture of water solutions of HM salts at

[2] P 2.1.10.1920-04 Guidelines for Health Risk. Assessment of Exposure to Chemical Substances, Polluting the Environment (Approved by the Chief State Sanitary Doctor of the Russian Federation on March 5, 2004).

[3] WRB (IUSS Working Group WRB. 2014. World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO. Rome.)

the rate of 10 mL/kg was applied to one of them and thoroughly mixed. The second part of the sample was moistened with an equal volume of distilled water. The obtained soil samples with the water content of about 60% of the field capacity were left for seven days at room temperature for the uniform distribution of water and HM salts. Then, samples S1 and S2 with added metal salts or water were divided into four parts (variants), one of which was a control for carbon-containing substances (biochar and lignohumate, separately and in combination) and HMs. The prepared soil samples (eight variants for each soil) were incubated for another seven days at room temperature.

Soil of each variant was placed into three pots of 3 L in volume (2.5 kg per each), from which samples were taken for chemical analysis and laboratory phytotesting. Seeds of mustard *Sinapis alba* L. (10 seeds per pot) were sown, and the pots were placed in a greenhouse with the mean air temperature of 16.8°C and the mean humidity of 61% for 30 days. The soil moisture in the pots was controlled by periodic weighing, after which distilled water was added. At the end of growing, plants were removed from the pots to determine their morphometric parameters and biomass, and soil samples were taken for chemical analysis and assessment of the chronic phytotoxicity.

We analyzed the following treatments for both soils (S1 and S2) during the experiment: C (control), L (lignohumate), B (biochar), LB (lignohumate and biochar), HMs (control with HMs), HML (HMs and lignohumate), HMB (HMs and biochar), and HMLB (HMs, lignohumate, and biochar).

Phytotesting was performed with the use of a green manure crop: white mustard (*Sinapis alba* L.). We evaluated acute phytotoxicity during the laboratory experiment and chronic phytotoxicity under greenhouse conditions.

The acute phytotoxicity of soil samples was assessed by the development of mustard seedlings in plastic plates according to the conventional method of measuring the biological activity of humic substances by the Phytoscan phytotesting approach (FR.1.39.2012.11560) [9], using the applicative and eluate methods.

According to the applicative method, the moistened (60% of the total water capacity) weighed portion of soil (60 g) was placed into the lower chamber of a two-chamber plastic tablet covered by one layer of filter paper, on which the plant seeds were put (ten seeds for each tablet, and three tablets for each variant).

The eluate method consisted in the assessment of the effect of water extract (eluate) from soil samples prepared by a conventional method (the soil to water ratio was 1 : 4). In this variant, three layers of filter paper soaked in water extract from soil samples (8 mL per each tablet) were placed into the lower chamber of the plastic tablet. The tablets were incubated at a temperature of 24 ± 2°C during 96 h. At the end of the exposure, the germination rate and root length of mustard seedlings were recorded. Seedlings in plates on filter paper moistened with distilled water were used as the control. The acute phytotoxicity was determined prior to and after the 30-day-long exposure of pots with plants. Changes in seed germination and in the length of the roots of mustard seedlings relative to those in the control were determined.

The chronic phytotoxicity of soils was assessed in pots according to ISO 22030-200. The duration of the exposure was 30 days; the temperature was from 22 to 28°C. We determined the germination, plant biomass (shoots and roots), and the growth and morphometric parameters of plants, as well as the accumulation coefficient of metals (the ratio of the element content in the dry biomass of the entire plant to its total content in soil).

Chemical analysis. Soil samples for the chemical analysis were dried at room temperature and ground in a mortar. We determined pH of water suspension, total carbon (ISO 14235:1998), total nitrogen (N_{tot}, using an Elementar EL III CNHS analyzer), ammonium nitrogen (GOST 26489-85, on a Hach DR 2800 photometer), nitrate nitrogen (PND F 16.1.8-98, using a Dionex ICS 2000 chromatograph), and available phosphorus and potassium compounds (according to Kirsanov on an Aglient 5110 ICP-OES spectrometer) in all variants of the experiment.

We determined the content of water-soluble copper, zinc, and lead in water extract (1 : 10) and their total content after decomposition of soil samples by aqua regia (a mixture of nitric and hydrochloric acids at the ratio of 1 : 3) and processing in a microwave. The HM content in plants was determined in dried and crushed samples treated with a mixture of nitric acid and hydrogen peroxide (7 mL/1 mL) and subjected to ozonation in a Milestone ETHOS D microwave oven (Milestone Laboratory, Agriculture/Food/Environment, Rev.0/2002). All measurements of HMs were performed by the ICP-OES method on an Agilent 5110 optical emission spectrometer (M-MVI-80-2008).

Statistical processing of data. The results were processed using a one-way ANOVA; the significance of differences was evaluated by the Tukey test ($p \le 0.05$). The final data were treated by the principal component method with variables characterizing the growth parameters of test plants in the pot experiment (root length, shoot length on the 30th day, dry biomass, and the number of flowers per plant), and the content of HMs in soils (the total content and water-soluble form). Calculations were performed in the statistical package Statistica 10 (StatSoft Inc., United States).

RESULTS

Chemical characteristics of soil samples. The chemical analysis of studied soils indicates that they differ significantly (by three times) in the organic matter content. Soil S1 is characterized by a high humus con-

Table 1. Agrochemical characteristics of agrosoddy-podzolic soils (0–20 cm) used in the experiment

Variant	C_{org}	N_{total}	C/N	pH	NH_4	NO_3	P_2O_5	K_2O	Cu*	Pb*	Zn*
	%				mg/kg						
High-fertile soil S1											
Control (C)	3.86	0.33	9.3	6.74	21.9	60.7	1685	701	22.0	23.7	89.1
Lignohumate (L)	4.07	0.34	9.3	6.77	12.3	48.9	1663	1059	23.4	23.9	93.3
Biochar (B)	7.74	0.32	43.5	6.75	11.3	61.8	1528	820	21.7	25.4	99.2
LB	6.08	0.35	28.3	6.75	12.7	32.0	1526	1104	22.9	22.7	95.1
HM	3.96	0.33	8.8	6.52	9.5	382	1684	743	485	515	1170
HML	3.5	0.36	10.0	6.10	8.3	453	1587	1075	669	830	1369
HMB	8.19	0.36	28.3	6.18	11.9	390	1679	789	563	526	1217
HMLB	8.73	0.36	29.5	6.33	5.4	403	1642	1164	601	705	1180
Low-fertile soil S2											
Control (C)	1.30	0.14	11.7	6.28	8.6	65.8	220	194	9.3	10.1	32.0
Lignohumate (L)	1.39	0.15	12.0	6.35	7.7	38. 6	270	464	10.9	9.2	31.6
Biochar (B)	6.53	0.15	24.2	6.41	8.2	48.3	228	286	9.4	9.3	39.7
LB	4.24	0.15	17.4	6.26	8.6	25.9	256	584	12.6	9.9	43.9
HM	1.40	0.16	12.0	5.07	19.7	433	205	259	596	712	1191
HML	1.40	0.14	9.7	5.43	14.1	403	218	429	600	826	1069
HMB	4.81	0.17	22.8	5.53	14.7	372	208	365	589	706	1111
HMLB	4.42	0.15	24.3	5.69	11.9	342	218	515	550	683	1090

* Total content; analytical error does not exceed 10%.

tent and is also well supplied with nitrogen, phosphorus, and potassium. On the contrary, the content of nutrients in the low-humus soil S2 is several times smaller (Table 1). These parameters determine soil fertility, as well as different buffer capacity and resistance to HM contamination, which is important for our experiment. In addition, the soils differ in the initial content of copper, lead, and zinc: it is higher in the carbon-rich soil (by two times for Cu and Pb and by almost three times for Zn). This testifies to higher rates of fertilizers applied to this soil and to its particular enrichment with elements contained in the fertilizers. Data on the contents of HMs indicate that both soils can be assigned to uncontaminated soils. Thus, the tested pollutants (copper, lead, and zinc) and carbon-containing substances (lignohumate and biochar) have been applied to initially non-toxic soils. According to hygienic regulations (GN 2.1.7.2511-09), the used rates of metals are five times higher than their permissible concentrations in soils of this type.

The application of HM salts has increased the content of zinc, copper, and lead by more than an order of magnitude; it has also caused some decrease in the soil pH.

Application of biochar has increased the organic carbon content by two to three times in soil S1 and by three to five times in soil S2 with a simultaneous two- to fourfold decrease in the organic matter enrichment with nitrogen (the C/N ratio).

Application of lignohumate has only slightly increased the contents of organic carbon and available potassium and also slightly affected other soil parameters (Table 1).

Chemical analysis of soil water extract. Water-soluble HM compounds are characterized by the highest mobility and availability for plants. Their contents can be judged from data on HMs concentrations in soil water extracts (Table 2). The results of the experiment indicate that, despite the strong soil contamination, only a small part of HMs is extracted by water (due to the low solubility of HM compounds and the high stability constants of HM complexes) The percentage of water extractable zinc is the highest.

Though the HMs have been applied to the soils at equal rates, their extraction by water from the low-fertile soil S1 is an order of magnitude higher than that from the high-fertile soil S2. This is related to the fact that the high organic matter content in the latter soil provides the formation of a higher number of sorption sites to bind HMs as compared with their sorption in the low-fertile soil.

The effect of carbon-containing products on the mobility of HMs under model contamination also depends on the soil fertility level. A slight decrease in the content of water-soluble compounds is observed in soil S1 only after 30 days of exposure and mustard cultivation. In soil S2, the mitigating effect of the remedi-

Table 2. Concentrations of HMs in water extracts, mg/kg

Variant	Prior to mustard growing			After mustard growing		
	Cu	Pb	Zn	Cu	Pb	Zn
High-fertile soil S1						
Control (C)	0.40	Bdl*	0.40	0.09	0.065	0.20
Lignohumate (L)	0.08	Bdl	0.70	0.10	Bdl	0.20
Biochar (B)	0.06	Bdl	0.60	0.08	Bdl	0.20
LB	0.09	Bdl	0.70	0.12	Bdl	0.40
HM	0.22	0.142	7.20	0.53	0.55	10.6
HML	0.49	1.701	14.6	0.54	0.23	8.10
HMB	0.19	0.189	8.20	0.35	0.33	5.60
HMLB	0.29	0.359	8.00	0.47	0.20	4.40
Low-fertile soil S2						
Control (C)	0.05	Bdl	0.5	0.05	Bdl	0.2
Lignohumate (L)	0.12	Bdl	5.3	0.06	Bdl	0.3
Biochar (B)	0.06	Bdl	0.5	0.05	Bdl	0.3
LB	0.06	Bdl	1.1	0.08	Bdl	0.4
HM	12.8	1.67	240	8.06	0.44	200
HML	6.3	0.34	179	6.33	0.56	191
HMB	8.4	0.51	199	4.92	0.57	174
HMLB	3.9	0.43	170	3.76	0.45	171

* Bdl—below detection limit of 0.0001.

ants is much more pronounced: the content of water-soluble forms of all the studied HMs decreases by 1.5–3 times.

Phytotoxicity assessment. The impact of HMs on soils with different organic carbon contents has been judged from data on the inhibition of germination and root length of *S. alba*.

Differences between the HM-contaminated samples of low-fertile and high-fertile soils are seen for both the applicative and eluate methods of the acute phytotoxicity test (Fig. 1).

In phytotesting by the eluate method, the eluate from the HM-contaminated sample S2 suppresses root growth by 21% relative to the control, while the eluate from sample S1 is nontoxic.

The differences between the soils according to the results of the applicative method of phytotesting are even more noticeable. Seed germination in soil S1 in the treatments with additives does not significantly differ from that in the samples without additives. In soil S2 contaminated with HMs, seed germination decreases by more than 50%. Additives of biochar applied individually and in combination with lignohumate somewhat improve seed germination, but the changes are not significant. The root growth suppression under the impact of HM contamination reaches 35% in soil S1 and 99% in soil S2.

The development of plants in the pot experiment confirms significant differences in the response of studied soils to HM contamination. Thus, HMs slow down the growth of plant roots by only 10% in soil S1 and completely suppress plant growth in pots with soil S2.

Any significant effect of lignohumate and biochar on the root development and seed germination in the three studied biotests is virtually absent, except for high-fertile soil S1 in the variant with a mixture of lignohumate and biochar, where their application has partly neutralized the toxic effect of HMs according to the results of the applicative method.

The response of test plants to polymetallic contamination in the pot experiment was additionally evaluated by a number of other standard test-parameters (ISO 22030:2009); differences between the treatments in the length of shoots, in the development of flowers and leaves, and in the biomass were determined (Fig. 2).

The length of plant shoots on uncontaminated samples of both soils does not significantly differ either on the 14th or on the 30th day (Figs. 2a, 2b). The application of HMs causes a significant inhibition of plant growth: by about 30% in soil S1 and by 100% in soil S2.

Similar changes are observed in the biomass. The application of lignohumate and biochar does not exert a significant effect on the length of shoots and on the biomass in both soils. There is no improvement in the

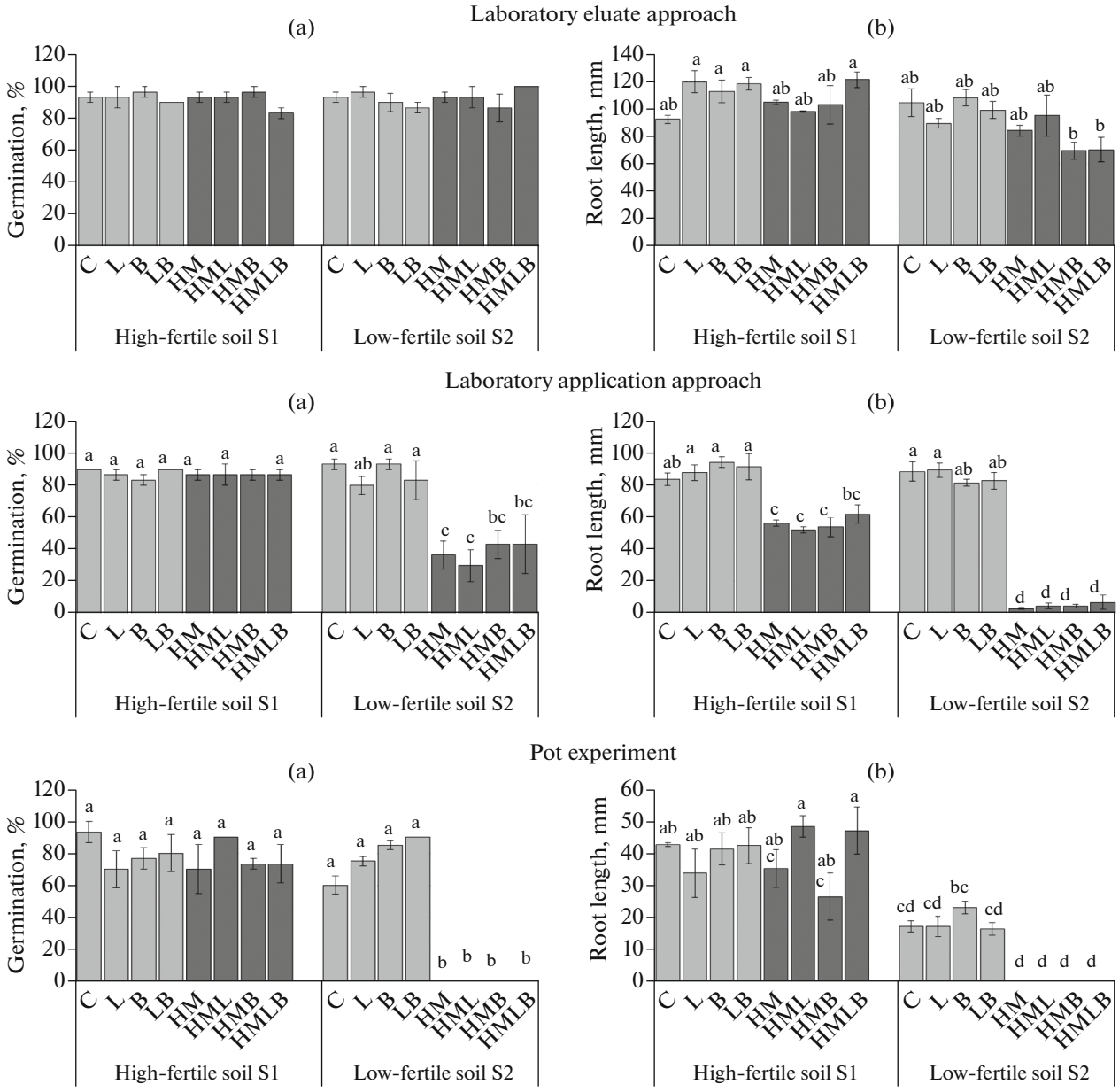

Fig. 1. Effect of polymetallic contamination and carbon-containing remediants on (a) seed germination and (b) root length of test plants *S. alba* in high-fertile (S1) and low-fertile (S2) soils upon the eluate, applicative, and vegetative phytotesting. Mean ± standard error ($n = 3$); values with different letters vary significantly ($p \le 0.05$, the Tukey test). C, control; L, lignohumate; B, biochar; LB, lignohumate and biochar; HM, heavy metals; HML, heavy metals and lignohumate; HMB, heavy metals and biochar; and HMLB, heavy metals, lignohumate, and biochar.

growth parameters and in the biomass after the application of lignohumate and biochar to both soils contaminated with HMs.

The applied HMs cause significant changes in the formation of plant leaves and completely suppress the formation of flowers on soil S1. The use of carbon-containing remediants does not reduce the toxic effect of HMs and does not improve these parameters.

The content of HMs in plants. Mustard plants grown on unpolluted soils are characterized by low Cu, Pb, and Zn contents (Table 3). The content of Cu and Pb in the plants increases 1.5 times after the biochar application, two times after the lignohumate addition, and 1.6 times after their combined use. An increase in the Zn content is less pronounced. Carbon-containing remediants applied to uncontaminated samples favor some accumulation of HMs in plant biomass, but their contents remain at the safe level.

Soil contamination by HMs results in their significant accumulation in plant biomass: the contents of

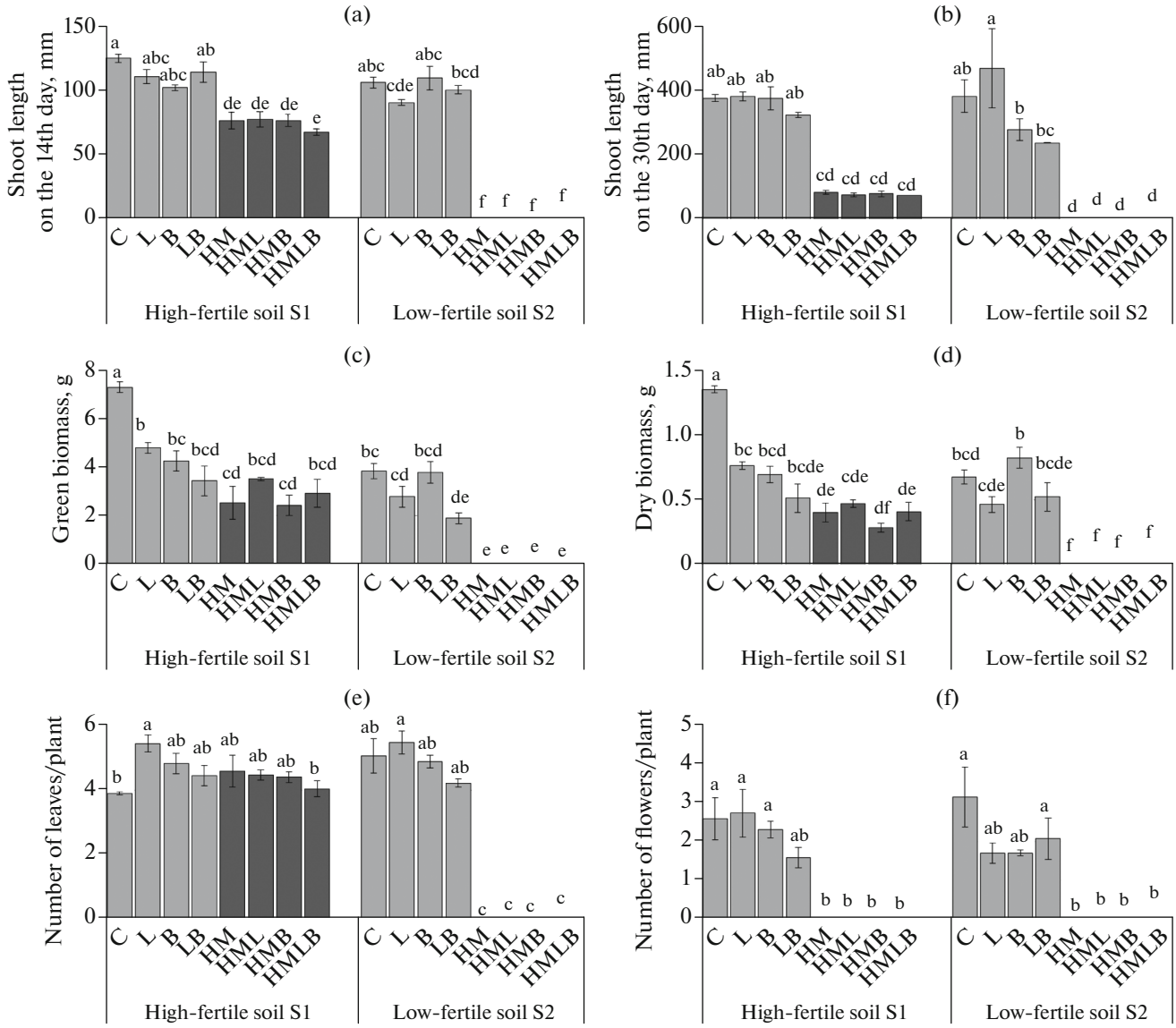

Fig. 2. The effect of polymetallic contamination and carbon-containing remediants on the (a, b) length of shoots, (c, d) biomass, and the number of (e) leaves and (f) flowers of test plants *S. alba* in high-fertile (S1) and low-fertile (S2) soils during phytotesting in the pot experiment. Mean ± standard error ($n = 3$); values with different letters vary significantly ($p \le 0.05$, the Tukey test). Designations of the variants are given in Fig. 1.

Cu, Pb, and Zn in plants increase by 20, 14, and 34 times, respectively.

The application of lignohumate to contaminated soil (HML) caused a decrease in the contents of Cu, Pb, and Zn in plants by 45%, 12%, and 28%, respectively, as compared to plants grown on contaminated soil without additives (HM). Biochar did not affect the accumulation of Zn and caused an increase in the contents of Cu (by 28%) and Pb (by 45%) in plants. In Biochar combined with lignohumate led to a decrease in the Zn content in plants by 9% and to an increase in the contents of Cu and Pb. Data on the coefficients of accumulation of Cu, Zn, and Pb by plants for the experimental variants on high-fertile soil S1 are presented in Table 4. It can be seen that the regularities of accumulation of HMs by mustard plants depend on the particular element. They are common for Cu and Pb: at the low element content in soil, the accumulation capacity of plants increases (which corresponds to published data [11, 26]), and both remediants enhance the accumulation of these metals by plants. On the contrary, the accumulation of Zn by mustard plants becomes higher with an increase in the Zn content of the soil. In the treatments with the high background content of all the three elements, their bioaccumulation decreases after the lignohumate application, whereas biochar does not display remediation effect.

Table 3. The content of HMs in mustard plants in different variants of the experiment, mg/kg

Variant	Cu	Pb	Zn	Cu	Pb	Zn
	high-fertile soil S1			low-fertile soil S2		
Control (C)	4.4	1.5	37.1	8.1	3.5	50.5
Lignohumate (L)	8.8	3.8	53.0	7.7	1.1	87.2
Biochar (B)	6.3	2.0	40.7	5.4	1.2	64.0
LB	7.3	1.7	47.2	6.5	1.1	68.7
HM	88	22.2	1260	Plants did not grow		
HML	48	19.6	910			
HMB	113	32.3	1291			
HMLB	149	47.3	1149			

Table 4. Coefficients of HM accumulation in the dry biomass of plants *S. alba* according to phytotesting of soil samples S1 in pot experiment

Control (C)	Cu	Pb	Zn
Lignohumate (L)	0.14	0.05	0.28
Biochar (B)	0. 29	0.10	0.35
LB	0.23	0.07	0.29
HM	0.22	0.06	0.37
HML	0.12	0.02	0.87
HMB	0.06	0.01	0.58
HMLB	0.15	0.03	0.91
Control (C)	0.21	0.05	0.73

The relationship between the phytotesting results and the chemical characteristics of soil. The principal component analyses (PCA) enables us to reveal and generalize regularities in the change of plant response to HMs in dependence on the soil properties.

It has been found that the first two principal components are the most significant (eigenvalues >1) and are responsible for 87% of the total variance of the experimental data (Fig. 3).

Soil properties—the presence of HMs (principal component (PC) 1) and the soil fertility status (humus content) (PC 2)—determine the parameters of plant development (the length of shoots and roots, biomass, and the number of leaves and flowers). We have not revealed the remediating effect of lignohumate and biochar on the samples of contaminated soils. In both

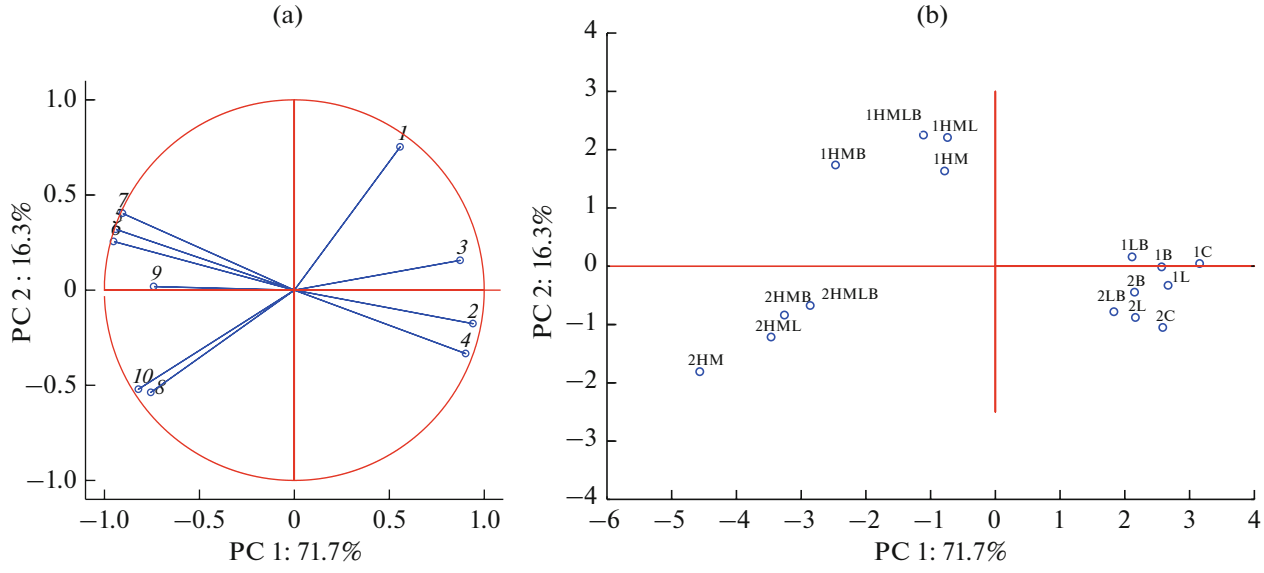

Fig. 3. Projection of (a) variables and (b) cases on the first and second principal components; (a): *1*—root length on the 30th day, mm; *2*—shoot length on the 30th day; *3*—dry biomass; *4*—the number of flowers per one plant; *5*—total Cu content in soil; *6*—total Pb content in soil; *7*—total Zn content in soil; *8*—Cu content in the extract; *9*—Pb content in the extract; *10*—Zn content in the extract; (b): designations are given in Fig. 1.

soils, HM-contaminated samples with and without remediants are assigned to the same groups.

The first principal component mainly reflects the gradient of changes in the content of HMs and in the parameters of plant development in the high-fertile and low-fertile soils, and the second principal component shows the relationship between the humus content and plant development parameters. A pronounced differentiation of soils with (left) and without (right) HMs is shown along PC1. The distribution of soils along PC2 is mainly related to the initial soil properties.

DISCUSSION

The phytotesting data indicate that agrosoddy-podzolic soils differing by three times in the organic carbon content and fertility status are characterized by pronouncedly different toxicity after their contamination with equal amounts of heavy metals (Cu, Pb, Zn). The same effect of the unfavorable humus status of soils on the increase in environmental risks under pronounced technogenic pollution has been shown in other studies [4, 11, 14, 16]. For example, on the third year after the contamination of high-fertile sandy soddy-podzolic soils, the mobility of Pb and Cd decreased by 37 and 25%, respectively, which resulted in a 1.6–2.4-fold drop in Pb and Cd accumulation in crops in comparison with that on the low-fertile soil [4].

Variations in the mobility of metals in soils with different organic carbon contents testify to a close relationship between toxicants and organic matter. The bioavailability of zinc, copper, and lead cations in the studied agrosoddy-podzolic soils with different organic carbon contents correlates well with the phytotesting results.

According to conventional methods, the toxicity analysis of water soil phase is performed by extraction (eluate) at the soil-to-water ratio of 1 : 4 [9, 32], which only imitates soil solution. However, this screening method is informative for the comparison of the bioavailability of toxicants. The toxic effect on root growth exerted by eluate of samples from the low-fertile soil (S2) contaminated with HMs is three times greater as compared to that in samples from the high-fertile soil (S1). The eluate of soil S1 contaminated with HMs exerts even a slight (13%) stimulating impact on root growth. This is related to the fixation of toxic metal cations on solid soil particles, which is evidenced by comparative data on the chemical composition of water extracts and soils. Phosphorus, which forms insoluble phosphates with HMs, may also contribute to a decrease in their availability in the high-fertile soil. Nutrients are extracted by water simultaneously with HMs. Their amount is greater in the high-fertile soil, so the toxic effect of metals in this variant is neutralized.

The eluate and applicative biotesting have been compared using samples of different soils (saturated alluvial meadow, plowed agrosoddy-podzolic, and typical soddy-podzolic) contaminated by toxicants [13, 14, 28]. The response of hydrobionts as test organisms for assessing the toxicity of eluates characterize the studied soils as significantly less toxic as compared to the response of test organisms being in direct contact with soil (earthworms *Eisenia fetida* and seeds of *Sinapis alba*) [14]. Such differences attest to the need to improve methods for assessing the toxicity of the liquid soil phase.

The results of the pot experiment (chronic toxicity bioassay) reflect the positive role of soil fertility management in detoxification of pollutants: HM contamination causes the inhibition of plant development in soil S1 by about 30% and leads to the death of plants in soil S2. The results of applicative laboratory tests and phytotesting in the pot experiment are well correlated ($k = 0.8$), whereas the results of eluate testing and phytotesting in the pot experiment are poorly correlated ($k = 0.1$).

Carbon-containing remediants are used to neutralize adverse effects and restore the ecological functions of soils. The cumulative effect of humic products and biochar on a decrease in the toxicity of soils contaminated by heavy metals under natural conditions has been described [28]. According to our data, the phytotoxicity of HMs depends on the soil humus content (fertility level) rather than on the application of carbon-containing remediants. In general, it may be concluded that a positive effect of lignohumate reducing the bioavailability of HMs is stronger than the effect of biochar. The efficiency of the latter is mainly seen in its combination with lignohumate (Table 4).

CONCLUSIONS

The status of biota under the same loads and different environmental conditions cannot be fully characterized only on the basis of data of chemical analyses. The tolerance of high-humus soils to heavy metals has been reported in many studies [4, 7, 8, 10, 11]. However, regulatory documents and methodological recommendations for soil toxicity assessment do not take into account different responses of test organisms to the impact of toxicants at different organic carbon contents in the initial soil. The assessment of soil pollution and monitoring of the content of heavy metals in soils are currently performed according to the regulatory document (hygienic normative) GN 2.1.7.2511-09 on tentatively permissible concentrations (TPCs of chemicals in soil. It regulates TPCs of HMs in soils taking into account only soil texture (sandy and loamy sandy and clay loamy and clayey soils) and acidity ($pH_{KCl} > 5.5$ or <5.5). Depending on these indicators, the TPCs of Cu, Pb, and Zn in soils vary by more than four times. Our data indicate that the threefold difference in the content of organic carbon in soils with the

same pH and texture leads to a significantly different response of plants to the same concentration of heavy metals. Therefore, differences in the humus content must be considered for establishing threshold values of the concentration of HMs in soils of the same type (agrosoddy-podzolic) having the same pH and some other physicochemical properties.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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