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Effect of Heavy Metals on the Enzymatic Activity of Haplic Chernozem under Model Experimental Conditions

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Abstract: The biological activity of soils is an essential parameter of their ecological status. The anthropogenic Heavy Metals (HMs) disturb the normal functioning of soil biota and, hence, the entire soil system. The aim of this work was to estimate the toxicity of loosely bound HM compounds in Haplic Chernozem using parameters of cellulose-degrading and urease activities. Contamination of Haplic Chernozem with Zn, Cd, Cu and Pb enlarged the group of mobile (loosely bound to soil) metal compounds, which includes exchangeable, complexed and specifically sorbed compounds of HMs. The increase of mobile HM forms content in soil inhibited the cellulose-degrading and urease activities after 30 days of the input of metal into the soil. A decrease in the toxic effect of metals on the studied parameters mainly occurred in the next 30 days. It was found that all HM forms similarly affected biological activity parameters. During two months from the moment of soil contamination with HMs, their toxic effect on cellulose-degrading and urease activities decreased. According to the degree of inhibition of biological activity in Haplic Chernozem, the studied metals formed the following series: Cd > Pb > Zn > Cu.

Keywords: Heavy Metals, Soils, Enzyme Activity, Pollution

Introduction

Soils, which are open buffer systems, need monitoring of their status under varying ecological conditions. Chemical contamination is frequently due to the input of Heavy Metals (HMs) into the soil. The specific ability of soil to sorb and distribute the arriving HMs among its components determines the state of the entire ecosystem of current interest is an objective estimation of the ecological status of soils, which is reduced to the revelation of factors determining the toxicity level of the contamination (Chaplygin *et al.*, 2014; Minkina *et al.*, 2015; 2017a; Hu *et al.*, 2014; Mandzhieva *et al.*, 2014). The biological activity of soils is an essential parameter of their ecological status. This parameter is characterized by a significant variation under the effect of diverse factors. The anthropogenic HMs disturb the normal functioning of soil biota and, hence, the entire soil system (Belyaeva, 2002; Gukalov *et al.*, 2015; Mineev *et al.*, 2008; Moreno *et al.*, 2009).

The current interest in the effect of HMs on soil microorganisms is related to the fact that the mineralization of organic residues, which ensures the integration of the biological and geological cycles, is mainly concentrated in the soil.

The use of bioindicators (cellulose-degrading and urease activities) is an objective and reliable approach to estimating the toxicity of HM forms in the soil. The biochemical and ecological conditions of pedogenesis, the intensity of biochemical processes in the soil and the fertility and biological activity of soil reflect the soil enzymes (Usman *et al.*, 2016; Naplekova and Stepanova, 1981; Nedyalkova *et al.*, 2017; Shlevkova, 1993). It was noted (Bardina *et al.*, 2013; Shipilina, 1996) that nitrifying and cellulose-degrading bacteria are the most vulnerable components of soil biota. Changes in their abundance can be indicative of the biological status of HM-contaminated soils. The activity of cellulose-degrading bacteria depends on the presence of mobile HMs in the soil and the degree of degradation of cellulose reflects the general direction of microbiological processes.

Changes in enzyme activity are indicators for the early diagnosis of negative changes in soil properties (Galstyan, 1982; Khaziev, 1982; Sardar, 2007; Vodyanitskii, 2014; Zvyagintsev, 2001). The inhibiting effect of HMs on enzymes is due to their complexation with proteins. Metal cations have a high affinity for the sulfhydryl groups of proteins, are coordinated with the imidazole nitrogen of histidine residues and carbonyl and carboxyl oxygen and form stable chelate bioclusters. Lead

cations are characterized by the displacement of the metal from the active site of the metalloenzyme (Ershov, 2005).

Model experiments (Kolesnikov *et al.*, 2006; 2013) revealed the following sensitivity series of enzymes to the effect of HMs in Haplic Chernozem: Urease > invertase > dehydrogenases > catalase. The determination of urease activity in soils is characterized by high informativity, sensitivity and specificity, as well as good reproducibility of results and, hence, low experimental error, high speed and wide distribution of the method, which provides comparable results. The inhibition of urease by HMs proceeds in two stages: Initial and prolonged inhibition. The initial inhibition shows the degree of inactivation of urease present in the soil (rapid response of the enzyme to contamination). The prolonged inhibition reflects a number of processes occurring in the soil such as the binding of metal cations by soil particles, including molecules of nutrition substrate, the inhibition of the microbiological fraction of soil, etc.

A direct correlation is observed between the content of HMs and the degree of changes in the biological properties of soil. According to the deterioration of biological properties under contamination with HMs, chernozems of southern Russia form the following series: Ordinary chernozem = Ordinary (northern-Azov) chernozem = Ordinary (Ciscaucasian) chernozem > typical (mountain) chernozem > typical (piedmont) chernozem > typical (Ciscaucasian) chernozem = Southern chernozem = Southern (chestnut) chernozem >> leached vertic chernozem (Kolesnikov *et al.*, 2013).

The effect of HMs on the biological activity of chernozemic soils was studied by many authors (Belyaeva, 2002; Kolesnikov *et al.*, 2006; 2014; Naplekova and Stepanova, 1981; Shlevkova, 1993). The distribution of HMs arriving into the soil and the effect of their toxic forms on soil biota are of interest. To estimate the toxicity of HMs in soils, it was proposed to analyze the group composition of their compounds using a combined fractionation scheme. This approach allows determining the mobility of HMs in soils from their distribution between the groups of compounds strongly and loosely bound to the soil. The group of loosely bound metal compounds includes exchangeable, complexed and specifically sorbed metal forms. The aim of this work was to estimate the toxicity of loosely bound HM compounds in Haplic Chernozem using parameters of cellulose-degrading and urease activities.

Materials and Methods

The object of study was deep low-humus heavy loamy calcareous ordinary chernozem (Haplic Chernozem) on loess-like loam. Soil was sampled in the Donskoe training farm located in the Oktyabr'skii district of the Rostov oblast (Russia). Soil samples were taken from a depth of 0-20 cm. Soil properties are given in Table 1.

Pots were filled with soil preliminarily sieved through a 1 mm sieve and contaminated with HMs; the

weight of soil in each pot was 3 kg. Solutions of Zn, Cd, Cu and Pb acetates were added to the soil separately. The water content was 60% of maximum water capacity; temperature was 20-22°C.

The experimental design included the contamination treatments ranked in order of Maximum Permissible Concentration (MPC) for total metals in the soil (100 for Zn, 55 for Cu, 32 for Pb, 0.5 mg/kg for Cd) (Artyushin *et al.*, 1992): (1) control without pollutant; (2) 1 MPC; (3) 2 MPC; (4) 5 MPC; and (5) 10 MPC. Experiments were performed in triplicate.

The ecological status of the studied soils was estimated from their biological activity parameters, total HM contents, HM retention strength and the content of loosely bound metal compounds.

The total HM contents were determined by X-ray fluorescence analysis. It was proposed to subdivide HMs in soils into two groups: Strongly and loosely bound to the soil (Minkina *et al.*, 2008). It is advisable to determine the content of HM groups in soils for estimating their mobilities and abilities of passing to adjacent environments. The group of loosely bound compounds includes exchangeable, complexed and specifically sorbed metal forms. The binding strength of metals by soil components was determined using the methods based on the parallel extraction of metal compounds with the following extractants:

- Ammonium acetate buffer solution (1 N $\text{CH}_3\text{COONH}_4$) (AAB) with pH 4.8 (soil: Solution = 1: 5; time of extraction 18 h). Metal compounds extracted by AAB are classified as exchangeable forms
- EDTA + AAB (1% solution of EDTA in AAB; soil: Solution = 1: 5; time of extraction 18 h). This solution presumably extracted exchangeable metal forms and those bound into organ mineral complexes. Consequently, the difference between the contents of metals in the (EDTA + AAB) and AAB solutions characterized the metal compounds bound into organ mineral complexes: So-called complex HM compounds
- 1 N HCl (soil: Solution = 1: 10; time of extraction 1 h). Along with exchangeable forms, this reagent solubilized specifically sorbed metal compounds, which mainly include metals relatively loosely retained by iron, aluminum and manganese oxides and hydroxides and carbonates. The difference between the contents of metals extracted by 1 N HCl and AAB is defined as specifically sorbed forms (Minkina *et al.*, 2008)

The content of metals in strongly bound compounds with organic and mineral components was calculated from the difference between the contents of total metals and their loosely bound forms in the soil. The relative content of loosely bound HM compounds in the soil characterized the mobility of the metal.

Table 1. Physicochemical and agrochemical properties of ordinary chernozem (0 - to 20 -cm layer)

Humus, %	pH	Physical clay, %	Clay, %	CaCO ₃ , %	CEC, cmol(+)/kg	Ca ²⁺ , cmol (+)/kg	Mg ²⁺ , cmol(+)/kg	Na ⁺ , cmol(+)/kg	K _{ex} , mg/kg	P ₂ O ₅ , mg/kg
6.6	7.2	48.1	28.6	0.1	371	310	45	1	228	16

Cellulose-degrading activity was analyzed by the application method from the degradation of linen fabric (Dobrovol'skaya *et al.*, 2015); the activity of urease was determined by the Khaziev (1990) method. The cellulose-degrading and urease activities were estimated 30 and 60 days after the contamination of soil with HMs. To estimate the activity of cellulose-degrading organisms, linen fabric was taken out of the soil after 30 days and the decomposition intensity of fabric was analyzed. New linen fabrics were placed in the same pots and analyzed after next 30 days to estimate the results of composting for post 30 days.

All analyzes were performed in triplicate. The interpretation of results was performed using variance and correlation–regression analysis.

Results and Discussion

The total contents of Pb, Cu, Cd and Zn in uncontaminated Haplic Chernozem did not exceed the MPCs for these elements except Cd Table 2. This could be related to the fact that the existing norms do not take into account the regional features of soils.

The content of total HMs is a capacity factor, which reflects the potential hazard of contamination for terrestrial ecosystems and characterizes the total content of elements in the soil but gives no idea of HM availability for plants and hydrobionts. The loosely bound HMs compounds are the most active nutrients for living organisms and contamination agents. The content of loosely bound HMs in the soil has a higher information value (Minkina *et al.*, 2017b).

Determination of loosely metal compounds in control variant showed that the contents of the exchangeable forms of the studied metals did not exceed of the MPC for Cu, Pb, Zn and Cd Table 2. According to the contents of exchangeable forms (% of total content) in the studied soils, the metals form the following descending series: Cd>Pb>Cu = Zn. At the addition of metals to the soil, the exchangeable HM content increased in 3 to 15 times (Table 2 and Fig. 1). The largest increase was noted at Pb (in 15.23 times). Under contamination conditions an increase in the proportions of complexed forms of HM in the group of loosely bound compounds was noted for all studied metals (Table 3).

The content of complex forms of HM in unpolluted soil was lower than that of exchangeable forms, with the exception of Cd. The number of exchangeable and complex forms of Cd was the same. According to the relative contents of complexed forms (% of total content) in soils without pollutants, the HMs form the following series: Cd > Pb > Cu = Zn. In all contamination treatments an increase in the proportions of complexed

forms of HM in the group of loosely bound compounds was noted for Cu and Pb Table 3, which is related to the capacity of these metals to form complex compounds with soil organic matter.

The content of specifically sorbed forms of HM in Haplic Chernozem was higher than exchangeable and complexed forms. In the studied unpolluted soil, the relative contents (% from total content) of specifically sorbed elements form the following series: Pb > Cu > Cd > Zn. At the addition of metals to the soil, the specifically sorbed HM content increased in 3.8 to 53.3 times Table 2. The specifically sorbed metals make up the highest proportion of loosely bound metal compounds in polluted and unpolluted and compose their potential reserve Table 3.

The initial contents of loosely bound metal forms in Haplic Chernozem were low. All of the studied metals predominantly occurred in the form of strongly bound compounds (83-93%). Consequently, 7-17% of HMs formed loosely bound compounds. In the uncontaminated background soil, Cd was the most mobile metal: Its contribution to the group of loosely bound compounds was 17%; Cu was less mobile: The share of its loosely bound compounds was 7%. In the group of loosely bound HM compounds, the share of specifically bound forms was 40-89%. The lowest content of specifically sorbed compounds was found for Cd, which suggests that this is the most mobile HM in soil, with consideration for the highest share of its loosely bound compounds (17% of the total metal content).

Contamination of soil with HMs increased the group of loosely bound compounds and affected the proportions of metal forms within the group. In the experimental treatments, the contents of loosely bound Pb, Cd, Cu and Zn compounds increased in accordance with their application rates.

The artificial contamination of soil with Pb increased the share of mobile metal forms already in the treatment with 2 MPC. At this application rate, the content of the group of loosely bound Pb compounds was 3 times higher than in the control. When the contamination of soil with lead increased, the metal mobility and, hence, the share of loosely bound compounds also increased. At the application rate of 5 MPC, the share of loosely bound Pb compounds exceeded that of strongly bound forms; in the treatment with 10 MPC, it reached 64%.

The increase in the content of loosely bound Pb compounds in the soil was mainly due to the increase in the share of the most mobile exchangeable and complexed metal forms. This was confirmed by the increase in the share of these forms in the group of loosely bound Pb compounds Table 3.

Table 2. Total contents, form distributions and relative contents of loosely bound HM compounds in

Experimental treatment	mg/kg				
	Total content	Exchangeable forms	Complexed forms	Specifically sorbed forms	Loosely bound forms % of total content
Pb					
Control	24.97	0.33	0.25	2.17	11
1 MPC	52.17	1.00	0.75	4.51	12
2 MPC	82.50	7.27	3.91	16.77	34
5 MPC	176.91	32.15	18.08	50.23	58
10 MPC	359.65	71.13	39.00	119.31	64
LSD ₀₉₅	12.18	0.86	1.19	3.30	
Cd					
Control	0.58	0.04	0.04	0.02	17
1 MPC	1.16	0.16	0.14	0.13	36
2 MPC	1.38	0.27	0.20	0.10	38
5 MPC	3.10	0.50	0.40	0.33	41
10 MPC	5.23	0.90	0.93	0.70	46
LSD ₀₉₅	0.56	0.25	0.13	0.11	
Cu					
Control	46.10	0.39	0.29	2.55	7
1 MPC	97.79	1.64	2.81	18.95	24
2 MPC	162.78	4.70	4.23	38.10	29
5 MPC	306.53	46.63	28.69	104.01	58
10 MPC	535.76	75.76	54.86	130.63	50
LSD ₀₉₅	18.95	1.39	0.83	12.27	
Zn					
Control	58.33	0.49	0.28	6.23	12
1 MPC	149.78	1.89	2.51	27.04	21
2 MPC	264.89	4.90	3.68	52.69	23
5 MPC	561.52	60.03	47.17	252.76	64
10 MPC	1041.90	153.21	114.91	331.95	57
LSD ₀₉₅	21.76	1.92	15.85	11.55	

Table 3. Distribution of HM forms in the group of loosely bound compounds in calcareous ordinary chernozem, %

Experimental treatment	Exchangeable forms	Complexed forms	Specifically sorbed forms
Pb			
Control	6.9	4.0	89.1
1 MPC	7.3	8.0	84.7
2 MPC	8.0	6.0	86.0
5 MPC	14.0	6.0	80.0
10 MPC	24.0	21.0	55.0
Cd			
Control	20.0	40.0	40.0
1 MPC	27.9	32.6	39.5
2 MPC	36.8	35.1	28.1
5 MPC	40.7	32.8	26.6
10 MPC	42.6	36.9	20.7
Cu			
Control	9.0	12.1	78.9
1 MPC	12.0	7.0	81.0
2 MPC	9.0	10.0	81.0
5 MPC	16.0	26.0	58.0
10 MPC	21.0	29.0	50.0
Zn			
Control	4.1	31.0	65.1
1 MPC	7.0	26.0	67.0
2 MPC	12.0	18.0	70.0
5 MPC	17.0	11.0	72.0
10 MPC	21.0	7.0	72.0

Cadmium is the most mobile element in the soil. The share of its mobile compounds in the control soil was 17%, including 20% of them in exchangeable forms and 40% in complexed forms. The contamination of soil with Cd was characterized by the most significant increase in its mobility. For example, an almost double increase in the content of loosely bound compounds was noted already at the metal application at 1 MPC Table 2. In the treatment with the application of 100 MPC, the share of loosely bound metal compounds increased in more than 7 times compared to the original soil. A significant increase in the share of exchangeable Cd forms was noted in the group of loosely bound compounds, which enhanced the metal mobility.

The total Cu content in the control soil did not exceed the MPC and corresponded to the background content of the element in Haplic Chernozem (Motuzova *et al.*, 2014; Mandzhiyeva *et al.*, 2014). The share of strongly bound Cu compounds was 93%. The application of Cu at a rate of 1 MPC increased the metal mobility: The share of loosely bound Cu compounds was 24% of its total content in the soil. The increase in the content of loosely bound Cu compounds correlated with the application rate of the metal and was mainly due to its complexed forms Table 2. A study of the fractional composition of Cu compounds in Haplic Chernozem (Minkina *et al.*, 2008) showed that organic matter is significantly involved in the sorption of this element by the soil. A reliable increase in the Cu content was observed already at the application of 1 MPC for complexed and specifically sorbed forms and 2 MPC for exchangeable forms. The increase in the content of mobile Cu forms compared to the total metal content at the application of 1-5 MPC determined the increase in the share of specifically sorbed forms.

The content of mobile Zn forms in the Haplic Chernozem was 13% of the total content of the metal. The application of Zn at a rate of 1 MPC doubled the content of its loosely bound compounds in the soil. When the contamination of soil with Zn increased, the contents of its mobile forms increased proportionally to the application rates mainly due to exchangeable and specifically sorbed forms Table 3 and Fig. 1. As in the case of Cu, a significant increase in the content of loosely bound Zn compounds was observed at the application of 5-10 MPC.

The contamination of soil with HMs resulted in a significant inhibition of the cellulose-degrading and urease activities of soil (Table 4). A reliable decrease in these parameters was noted already at a contamination rate of 1 MPC. An exclusion was provided by the treatment with the application of Zn at 1 MPC, where some increase in urease activity was noted for the incubation during 30 days. For cellulose-degrading activity, the HM contamination of soil was more toxic at the incubation for 30 days. The incubation during 60 days can be characterized by adaptation processes. For cellulose-degrading activity, adaptation processes were more manifested than for urease

activity. The cellulose-degrading and urease activities of Haplic Chernozem were more inhibited by the excess amounts of Cd and, to a lesser extent, Cu in the soil.

At the contamination of soil with an HM, the decrease in cellulose-degrading and urease activities in most treatments correlated with the content of the added HM. The level of soil contamination with HMs at a rate of 1 MPC after composting for 30 days decreased the cellulose-degrading activity by 17 to 35% compared to the control. After the incubation for 60 days, this parameter varied from 7 to 23%. For urease, a decrease in activity by 15-17% was noted after 30 days in the treatment with 1 MPC; after 60 days of incubation, the decrease was 9-23%. After incubation for 60 days, cellulose-degrading activity increased by 22, 23, 10 and 25% in the treatments with the application of Zn, Cd, Cu and Pb, respectively.

The urease activity of soil was instable in the treatment with the application of 1 MPC. For example, an increase in urease activity compared to the control was noted for Zn at the composting for 30 days, as well as a decrease by 20% after 60 days of composting. According to some authors, low HM concentrations can activate the enzyme activity (Belyaeva, 2002; Grigoryan, 1990; Minnikova *et al.*, 2017). Under Cd contamination, the activity of urease decreased by 17% after 30 days and returned to the control level (23%) after 60 days. Similar changes were revealed at the application of Cu and Pb. The activity of urease in the treatment with 1 MPC decreased by 15% compared to the control after 30 days of incubation and increased by 5-6% after 60 days.

The contamination of Haplic Chernozem with HMs at a rate of 10 MPC resulted in a higher decrease in cellulose-degrading activity and strongly depended on the metal nature. For example, the activity of cellulose-degrading organisms in the treatment with the addition of Cd decreased by 75% after 30 days of composting. However, this parameter increased by 16% after 60 days. An increase in activity of 16, 8 and 2% was also observed for Zn, Pb and Cu, respectively. The urease activity of soil in this treatment was manifested differently. Further decrease in activity from 22% after 30 days to 41% after 60 days was noted for Zn. A less significant decrease in urease activity was noted for the other studied metals: By 9% for Cd, by 4% for Pb and by 3% for Cu. Among all of the studied metals, Cd inhibited the biological activity of soil to the lowest degree.

Comparison of the contents of exchangeable, complexed and specifically sorbed HM compounds in the soil with the cellulose-degrading and urease activities revealed close inverse correlations (Table 5). The relationship between the cellulose-degrading activity and the content of mobile HM forms in the soil depended on the metal nature. The closest correlation with the studied parameters was revealed for Zn and Cu. Correlation analysis showed that all of the studied HM forms had an inhibiting effect on parameters of soil biological activity.

Table 4. Effect of HMs on the biological activity of soil, in % of the control

Experimental treatment	Cellulose-degrading activity				Urease activity			
	Zn	Cd	Cu	Pb	Zn	Cd	Cu	Pb
Composting for 30 days								
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1 MPC	72.0	65.0	83.0	67.0	109.0	83.0	85.0	85.0
2 MPC	70.0	57.0	79.0	62.0	95.0	74.0	78.0	80.0
5 MPC	57.0	46.0	72.0	53.0	88.0	69.0	72.0	65.0
10 MPC	46.0	25.0	62.0	39.0	78.0	42.0	58.0	52.0
LSD ₀₉₅	6.5	5.0	6.4	3.9	4.8	7.1	6.3	8.8
Composting for 60 days								
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1 MPC	94.0	87.0	93.0	92.0	80.0	77.0	90.0	91.0
2 MPC	86.0	70.0	87.0	81.0	70.0	76.0	83.0	81.0
5 MPC	74.0	56.0	78.0	77.0	64.0	60.0	77.0	59.0
10 MPC	62.0	41.0	64.0	47.0	59.0	51.0	61.0	56.0
LSD ₀₉₅	4.1	4.8	6.7	3.9	4.3	7.0	7.1	4.8

Table 5. Correlation coefficients between heavy metal exchangeable form content in soil and biological activity

Element	Forms	Incubation for			
		Cellulose-degrading activity		Urease	
		30 days	60 days	30 days	60 days
Zn	Exchangeable	-0.92	-0.91	-0.89	-0.92
	Complexed	-0.95	-0.93	-0.87	-0.93
	Specifically sorbed	-0.96	-0.89	-0.82	-0.93
Cd	Exchangeable	-0.75	-0.91	-0.83	-0.90
	Complexed	-0.74	-0.92	-0.90	-0.92
	Specifically sorbed	-0.72	-0.90	-0.88	-0.87
Cu	Exchangeable	-0.93	-0.76	-0.61	-0.84
	Complexed	-0.94	-0.75	-0.59	-0.86
	Specifically sorbed	-0.94	-0.86	-0.63	-0.87
Pb	Exchangeable	-0.78	-0.90	-0.40	-0.71
	Complexed	-0.79	-0.90	-0.40	-0.78
	Specifically sorbed	-0.84	-0.94	-0.41	-0.63

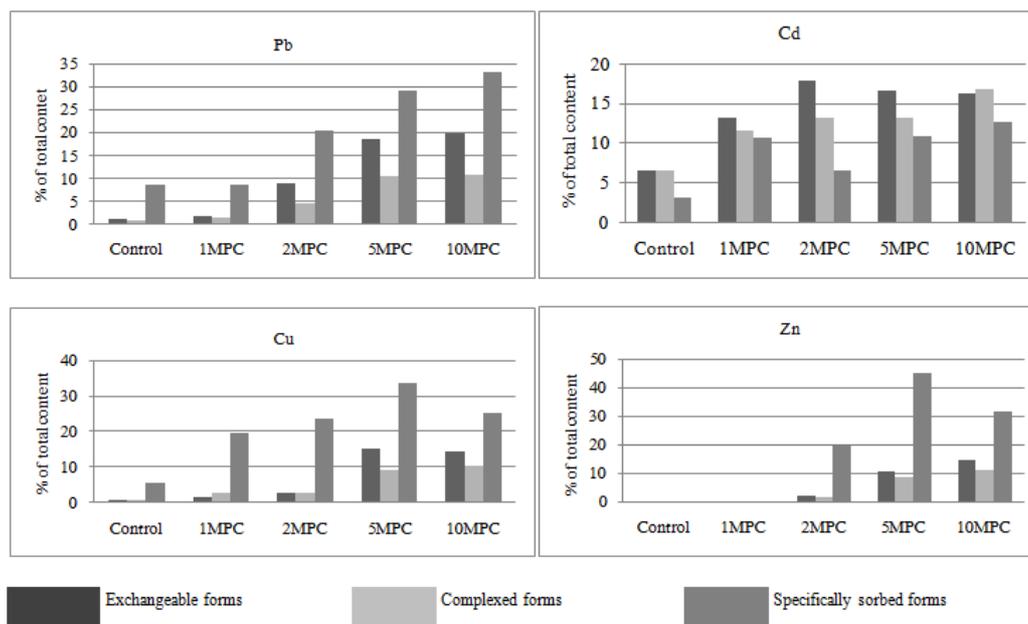


Fig. 1. Relative contents of loosely bound (exchangeable, complexed and specifically sorbed) HM forms in calcareous ordinary chernozem

Conclusion

- In the uncontaminated Haplic Chernozem, a larger part of the Zn, Cu, Cd and Pb is firmly fixed by the soil components. The portion of loosely bound HM which includes exchangeable, complexed and specifically sorbed metal forms is 7-17% of their total contents
- Contamination of Haplic Chernozem with Zn, Cu, Cd and Pb increased the content of loosely bound metal compounds. The largest contribution was made by exchangeable and complexed metal forms. Cu was less mobile and Cd was the most mobile metals in the soil
- The increase in the content of loosely bound compounds of Zn, Cu, Cd and Pb in the soil resulted in a stable inhibition of cellulose-degrading and urease activities of soil during 30 days after the application of metals into the soil. A decrease in the toxic effect of metals on the studied parameters mainly occurred in the further 30 days
- By the degree of cellulose-degrading and urease activities inhibition, heavy metals formed the following series: Cd > Pb > Zn > Cu

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Author Contributions

Natalia Gromakova and Saglara Mandzhieva: Participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

Tatiana Minkina, Galina Motuzova and Olga Birukova: Coordinated the data-analysis and contributed to the writing of the manuscript.

Sergey Kolesnikov and Natalia Barsova: Contributed in drafting the manuscript and reviewing it critically for significant intellectual content

Victor Chaplygin: Participated in all experiments, collected the field data performed the statistical analysis.

Inna Zamulina and Yurii Litvinov: Analyzed the soil, performed the statistical analysis.

Svetlana Sushkova: Collected the field data and contributed to the writing of the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of other authors have read and approved the manuscript and no ethical issues involved.

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