

Influence of heavy metals pollution on the formation of microbial community in gray forest soil

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The state of microbial communities of gray forest soil contaminated with heavy metals at a dose of 5, 10, 100 MPC, in the presence and absence of vegetative corn biomass has been investigated in model experiments. The protective function of the phytocenosis concerning several groups of microorganisms, in particular, azotobacter and polysaccharide-synthesizing bacteria, has been shown. The number of azotobacter in the rhizosphere of plants exceeds the indices of soil without plants: in the control by 3.33 %, at 5 MPC – 36.6, at 10 MPC - by 95.6 %. The indicative function of azotobacter has been confirmed concerning soil pollution with heavy metals. The number of azotobacter is decreased with increasing the pollutant dose in the soil without plants: at 5 MPC - by 2.64 times, at 10 MPC - by 6.67 times, the corresponding indicators for plants rhizosphere are 2.05 and 3.52 times. Azotobacter is not detected by the method of overgrowing soil lumps at the maximum level of soil contamination with heavy metals (100 MPC). The mycelial forms of microorganisms (micromycetes) are highly resistant to contamination with heavy metals that have been confirmed. The activity of mineralization of organic substances, including hummus, is decreased with the increasing of pollutant dose in the soil has been shown. The pedotrophy index decreases at 5 MPC by 3.16%, at 10 MPC – 30.9, and 100 MPC - by 46.8 % in soil without plants. Similar numbers for variants with plants fluctuate between 14.2 and 105.4 %. The conclusion about a lesser susceptibility to microbial mineralization of complexes of humic acids and heavy metals has been confirmed. With an increase in the dose of the pollutant, the activity of humus mineralization decreases in soil without plants: at 5 MPC - by 16.7 %, at 10 MPC – 12.6, at 100 MPC - by 74.7 %; with plants: at 5 MPC - by 14.3 %, at 10 MPC – 8.33, at 100 MPC - by 113.7 %. It is also drawn attention to the fact that the activity of humus mineralization in the soil rhizosphere is lower than in the soil without plants. In our opinion, the reason for this is the presence of easily utilized substrates in the composition of plant root exudates. It makes the mineralization of hard-to-reach humus molecules inappropriate.

Keywords: microbiocenosis; gray forest soil; heavy metals; azotobacter; polysaccharide-synthesizing bacteria; micromycetes; mineralization; phytotoxicity

Introduction

The problem of environment, soil and food pollution with heavy metals is one of the most urgent today. The number of publications in the scientific literature on this topic is only inferior to the most studied problem - fixation of atmospheric nitrogen by prokaryotic organisms. The studies of complex systems, which are included not only the soil and microorganisms living in it, but also plants that are directly effected on the formation of microbial communities under conditions of soil pollution with heavy metals have been actualized recently (Epelede et al., 2010; Xiong et al., 2010). Modern methods such as measuring of the basal respiration level, substrate-induced respiration, studying of the communities' structure by the PCR analysis and the distribution of functional genes are used to assess the response of microorganisms to contamination with heavy metals. The aim of our research was to study the changes of microbial community functional structure of gray forest soil under the influence of increasing concentrations of heavy metals.

Materials and Methods

The model experiment was established with the using of gray forest large-silty light-loamy soil of the stationary experiment of the National Scientific Center "Institute of Agriculture of NAAS" (experimental farm "Chabany", Kiev-Svyatoshinsky district, Kiev region). The soil of the "intensive agriculture soil" option, which includes a field crop rotation with a saturation of mineral fertilizers of N96P108K112.5 based on the plowing background of crop by-products (2010 crop - oats) was used. The 0-20 cm soil layer contains: humus – 1.75 %, alkaline hydrolyzed nitrogen (according to Cornfield) – 6.86 mg, nitrate nitrogen – 6.46, ammonium nitrogen – 0.20, mobile phosphorus – 60.0 mg and exchangeable potassium – 25.4 mg per 100 g of air dry soil, pH (KCl) – 4.9. The soil was sampled in the fall and its biological activity was restored by moistening in thermostat at 25 °C for 21 days before carrying out a model experiment. Variants with artificially created backgrounds of zinc and lead were investigated: No 3 and 4 - 5 times excess of MPC; No 5 and 6 - exceeding the MPC by 10 times; No 7 and 8 - exceeding the MPC by 100 times. The control sample was the soil with a natural concentration of heavy metals. The concentration of metals was calculated using the acid-soluble fraction at the creating the background pollution, since it is this fraction is considered the main technogenic component of the stock of heavy metals in the soil. The control vessels were filled with a KNO₃ solution at an appropriate concentration is to equalize the nitrogen content. Maize seeds were sown in half of the vessels 8 days before the introduction of heavy metals. The corn plants were in the 3-4-leaf phase by the time the heavy metals were added. The state of microbial communities was studied 32 days after the addition of heavy metals. The number of microorganisms of individual ecological, trophic and functional groups was assessed by inoculation a soil suspension on appropriate nutrient media (Tepper et al., 2004). The intensity indices of mineralization processes of nitrogen- and carbon-containing compounds, phytotoxic properties of the soil were determined in accordance with the previously described (Malynovska et al., 2011).

Results and Discussion

Plants with their root secretions act as protectors against microorganisms of several ecological and trophic groups, in particular, azotobacter. The number of azotobacter in the rhizosphere of plants exceeds the parameters of soil without plants: in the control by 3.33 %, at 5 MPC - 36.6, with 10 MPC - by 95.6 % (Table 1). The higher the level of soil contamination with heavy metals, the more pronounced the protective function of plants relative to azotobacter. Azotobacter belongs to microorganisms - indicators of the ecological purity of the soil, from our point of view. Its number is decreased when the soil is contaminated with both oil products and heavy metals (Malynovska et al., 2011; Malynovska et al., 2012). The number of azotobacter decreases with the increasing of pollutant dose in soil without plants: at 5 MPC - by 2.64 times, at 10 MPC - by 6.67 times in the presented data. The corresponding indicators for the plants rhizosphere are 2.05 and 3.2 times. Azotobacter is not detected by the fouling of soil lumps at the maximum level of soil contamination with heavy metals (100 MPC). We showed earlier, when studying gray forest soil in a state of fallow that not only the abundance of azotobacter changes under the influence of a dose of heavy metals, but also the physiological and biochemical activity of its cells significantly decreases under the influence of increasing doses of heavy metals (Malynovska et al., 2012). Thus, the number and physiological and biochemical activity of azotobacter cells are diagnostic indicators of the intensity of contamination of gray forest soil with heavy metals has been shown in a series of model experiments (Malynovska et al., 2012; 2013; 2013a).

Table 1. Influence of heavy metals on the number of microorganisms in gray forest soil contaminated for 32 days (intensive agriculture soil, million CFU*/g of dry soil).

Variant	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control without phytocenosis	445.0	127.0	50.4	60.0	124.0	0.094	145.1	170.2	5.64	16.8	16.2	0.169	18.4	3.38
Control + phytocenosis	658.9	123.4	68.3	62.0	167.7	0.048	174.9	111.8	7.99	18.2	15.6	0.671	36.3	10.0
5 MPC** without phytocenosis	397.0	145.9	40.1	22.7	51.0	0.049	125.5	231.0	1.89	12.5	12.5	0.174	7.56	2.65
5 MPC + phytocenosis	669.0	103.3	54.0	31.0	152.3	0.102	154.9	90.3	8.99	14.1	10.2	0.859	9.06	21.8
10 MPC without phytocenosis	410.0	130.0	33.3	9.00	123.6	0.041	102.3	163.0	1.50	10.5	9.37	0.127	9.37	1.50
10 MPC + phytocenosis	430.2	130.5	53.4	17.6	148.5	0.113	125.2	170.1	10.6	12.0	4.95	0.336	15.6	14.1
100 MPC without phytocenosis	403.7	131.8	33.3	0	0.010	0.042	89.5	213.5	0.761	5.94	26.8	0.388	52.1	0.381
100 MPC + phytocenosis	842.3	144.1	150.3	0	0.799	0.032	147.4	212.3	3.21	6.85	8.41	0.865	10.8	4.81
SSD05	6.20	4.00	2.54	1.95	2.10	0.005	9.15	6.55	0.72	1.65	0.58	0.115	0.30	0.15

1 - Ammonifiers, 2 - Immobilizers of mineral nitrogen, 3 - Oligonitrophils, 4 - Azotobacter, % of soil lumps fouling, 5 - Denitrifiers, 6 - Nitrifiers, 7 - Pedotrophs, 8 - Cellulose-destructive, 9 - Polysaccharide-synthesizing, 10 - Autochthonous, 11 - Actinomycetes, 12 - Micromicetes, 13 - Mineral phosphate mobilizers, 14 - Acid-forming. CFU * - colony-forming unit, MPC ** - maximum permissible concentration.

The number of acid-forming microorganisms is much higher in the plants rhizosphere in comparison with the soil without phytocenosis: without contamination - 2.96 times, with 5 MPC - 8.23, with 10 MPC - 9.40, with 100 MPC - 12.7 times (Table 1). Organic and mineral acids take an active part in the dissolution of mineral elements from their poorly soluble forms. This fact can be considered the evidence of the regulation of the number of acid-forming microorganisms by plants and, consequently, the intensification of the process of transferring mineral elements into a state accessible to plants. Soil contamination with heavy metals, for an unknown reason, enhances this process, which is also confirmed by the studies carried out by us using gray forest soil in a state of fallow (Malynovska et al., 2012). The number and physiological-biochemical activity of polysaccharide-synthesizing bacteria depend on the level of soil contamination with heavy metals has been shown earlier, using the example of a gray forest soil deposit. In particular, the number of polysaccharide-synthesizing bacteria increases in the rhizosphere of plants at 5 MPC - 2.24 times, at 10 MPC - 6.89, at 100 MPC - 1.67 times (Malynovska et al., 2012). We explained this by the protective function of bacterial polysaccharides relative to producer cells. We obtained somewhat different patterns when studying the contamination of gray forest soil with heavy metals for the same period (32 days), however, using soil that is in intensive agricultural crop rotation (see research methods). Therefore, the number of polysaccharide-synthesizing bacteria, in soil without plants, are decreased proportionally the dose of heavy metals: at 5 MPC - 2.98 times, at 10 MPC - 3.76, at 100 MPC - 7.41 times (Table 1). However, the number of polysaccharide-synthesizing bacteria increases in the rhizosphere of plants, with an increase of the pollutant dose, as in the case of fallow land. At the same time, the regularity of the influence of plant cultivation is clearly traced: the number of polysaccharide-synthesizing bacteria is always higher in the rhizosphere than in soil without plants, and the higher the level of contamination with heavy metals, the greater the degree of influence of plants on the number of bacteria. Previously, it was also found that the number of polysaccharide-synthesizing bacteria decreases with an increase of heavy metals dose in short-term soil contamination (one day), since the synthesis of exopolysaccharides has not yet become a selective advantage of bacteria of this group (Malynovska et al., 2013). Thus, the number of polysaccharide-synthesizing bacteria depends on the period of soil contamination, the dose of heavy metals, the presence or absence of plants, as well as the method of use (fallow, extensive or intensive agricultural soil) and agrochemical characteristics of the contaminated soil. Bacterial polysaccharides

have protective functions against the action of heavy metals on bacterial cells (Dudman, 1977); however, the degree of manifestation of this function depends on the characteristics of the soil that has been contaminated.

The data published in the scientific literature on the effect of heavy metals on soil microorganisms are often contradictory. The discrepancy between the patterns, identified by the researchers, may be due to different methods, different doses of heavy metals, different compositions of media for the cultivation of one or another group of microorganisms, and even different terminology. Therefore, Zvyagintsev et al. (1987) concluded that pollution with heavy metals significantly reduces the number of oligonitrophils in sod-podzolic soil. At the same time, MPA medium diluted 100 times was used to determine the amount of oligonitrophils, while Mishustin's medium with leached agar (to remove residual nitrogen compounds) is the generally accepted medium for the cultivation of oligonitrophils. Our data indicate that oligonitrophils do not belong to microorganisms sensitive to heavy metals in both short and medium term contamination (Malynovska et al., 2012; 2013; 2013a). According to Chugunova et al. (1990), nitrifies and cellulolytic are the most sensitive to the inhibitory action of heavy metals. According to our data, the number of nitrifies decreases significantly only at the highest dose of heavy metals studied - 100 MPC (Table 1). The number of cellulolytic also depends largely on the presence of plants or the remains of their roots than on the dose of heavy metals.

Table 2. Indicators of the intensity of mineralization processes and phytotoxic properties of gray forest soil (intensive agriculture soil) at different levels of contamination with heavy metals.

Variant	Pedotrophy index	Oligotrophy coefficient	Nitrogen mineralization coefficient	Humus mineralization activity,%	Soil respiration intensity, mg CO ₂ kg soil	Weight of 100 plants of test culture - winter wheat, dry weight, g		
						stems	roots	total weight
Control without phytocenosis	0.326	0.113	0.285	11.6	99.2	14.3	5.26	19.6
Control + phytocenosis	0.265	0.104	0.187	10.4	777.6	14.7	6.86	21.6
5 MPC without phytocenosis	0.316	0.101	0.368	9.94	87.3	11.7	5.00	16.7
5 MPC + phytocenosis	0.232	0.081	0.154	9.10	406.8	11.5	6.69	18.2
10 MPC without phytocenosis	0.249	0.081	0.317	10.3	91.3	12.4	4.73	17.1
10 MPC + phytocenosis	0.291	0.124	0.303	9.60	431.8	10.9	7.12	18.0
100 MPC without phytocenosis	0.222	0.082	0.327	6.64	87.9	The seeds of the test culture did not germinate due to the high toxicity		
100 MPC + phytocenosis	0.175	0.178	0.171	4.65	290.9			
SSD05	0.010	0.008	0.011	0.120	3.14	0.40	0.52	

Some researchers consider mycelial forms of microorganisms is more resistant to the action of heavy metals (Zvyagintsev et al., 1997; Selivanovskaya et al., 2002). This conclusion is confirmed both for options without plants, and for growing corn (Table 1) according to the results of our studies. The number of CFU of micromycetes increases especially strongly at 100 MPC; it exceeds the number of fungi in the control without plants by 2.3 times, with plants - by 1.29 times. The number of representatives of another mycelial form - actinomycetes - decreases with an increasing of heavy metals dose, maximum - at 2-3 times. At the same time, the number of actinomycetes decreases more sharply than the total number of prokaryotes, which decreases at 5 MPC by 12.5 %, at 10 MPC - 17.8, at 100 MPC - by 23.4 % (without plants).

Heavy metals slow down (inhibit) the mineralization of organic matter in the soil according to well-established ideas and the results of our own research (Malynovska et al., 2012; El-Shinnawi et al., 1976; Landa et al., 1978). The obtained data confirm this pattern: the pedotrophy index decreases in soil without plants at 5 MPC by 3.16 %, at 10 MPC - 30.9, and at 100 MPC - by 46.8 % (Table 2). Similar numbers for variants with plants fluctuate between 14.2 and 105.4 %.

M.Ya Stepanova (1976) showed that the content of heavy metals in soils correlates with the humus content. It is possibly due to the formation of complexes between heavy metal ions and molecules of humic acids, which are less susceptible to mineralization than molecules of humic acids outside the complexes. An increase of the humus content in soils contaminated with heavy metals was noted in the works of Bezuglova et al. (1999), which the authors associate with the negative influence of pollutants on the state of microbial cenosis and a decrease in the overall biological activity of the soil. Gamaley et al. (2008) also confirmed this conclusion on the example of clear gray and chernozem soils of roadside strips of highways. They showed qualitative differences between the humus of polluted and relatively clean soils: in the soils of roadside strips, humus is formed, enriched with more soluble humic compounds, which have greater mobility and more pronounced acidic properties.

Our data confirm the conclusion about a lesser susceptibility to microbial mineralization of complexes of humic acids and heavy metals (Table 2). The activity of humus mineralization are decreased with the pollutant dose increasing in soil without plants: at 5 MPC - by 16.7 %, at 10 MPC - 12.6, at 100 MPC - by 74.7 %; with plants: at 5 MPC - by 14.3 %, at 10 MPC - 8.33, at 100 MPC - by 113.7 %. The

activity of humus mineralization in the soil rhizosphere is lower than in the soil without plants. In our opinion, the reason for this is the presence of easily utilized substrates in the composition of plant root exudates, which makes the mineralization of hard-to-reach humus molecules inappropriate. The data obtained by us do not agree with the results of the studies of M.A. Postnikova (2007). According to this research, the dehumification process, associated with the activity of the bacterial complex, occurs more intensively in soil contaminated with readily available organic compounds (the phenomenon of co-metabolism). Probably, the intensity of the humus mineralization process is determined by this. The regularities of its mineralization, because of the lack of mineral elements (primarily nitrogen), will differ from the regularities of humus mineralization as a substrate, i.e. source of carbon and energy, as it was the case in Postnikova's model experiments.

Contamination with heavy metals at a dose of 5-10 MPC leads to a significant increase of the soil phytotoxicity without phytocenosis (by 14.6-17.4%), with phytocenosis - by 18.7-20.0% (Table 2). The dose of heavy metals in 100 MPC is so toxic that the seeds of the test culture do not germinate.

Conclusion

Structural and functional changes are occurred in the microbial community of gray forest soil during medium-term contamination of heavy metals, directly influenced the soil microorganisms and indirectly the plants. The magnitude and direction of such changes depend on the level of soil contamination, the presence of vegetation cover and the way of soil using. The mineralization of organic and organomineral complexes, including humus, is slowed down with the increasing of the pollutant dose in the soil, and, on the contrary, the accumulation of toxic substances is activated.

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