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Enzymatic activity of podzolized chernozem contaminated by pollutants during its detoxification

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Abstract. The soil is an indicator of the general technogenic situation. In terms of the scale of pollution, as well as the impact on biological objects, heavy metals occupy a special place among pollutants. One of the priority pollutants are Pb, Cd, Zn, Cu. In assessing the ecological state of the environment, the study of the soil cover plays an important role. The most informative integral characteristics of the biological activity of the soil is the activity of soil enzymes. In a lysimetric experiment with podzolized chernozem, we studied the change in the biological activity of soil in terms of dehydrogenase, catalase, urease, invertase and phosphatase enzymatic activity under the complex influence of heavy metals under conditions of the use of detoxification agents. The soil at the experimental site had the following characteristics: pH_{KCl} 6.2; humus content - 3.2%, P_2O_5-229 mg kg^{-1}, K_2O-250 mg kg^{-1} of soil. Organic and mineral fertilizers in various combinations were used as detoxifiers. According to the obtained data, the redox enzyme - dehydrogenase and hydrolytic enzymes urease and phosphatase are the most sensitive to soil pollution. The best decontamination effect is obtained when using a system of organo-mineral fertilizers, what contributes to an increase in the activity of soil urease by 3.38 times, invertase - by 2.47 times, phosphatase - by 1.48 times, dehydrogenase - by 1.46 times, catalase - by 1.60 times. Changes in the activity of these enzymes can be used to diagnose the effectiveness of the use of various fertilizer systems on soil contaminated by heavy metals.

Key words: podzolized chernozem, detoxification, heavy metals, dehydrogenase, catalase, urease, invertase, phosphatase.

INTRODUCTION

Heavy metals that enter the environment as a result of human production activities (industry, transport, etc.) are one of the most dangerous pollutants. These metals tend to be fixed in individual links of the biological cycle, accumulate in the biomass of microorganisms and plants and enter the body of animals and humans through trophic chains, negatively affecting their vital activity (Gromakova et al., 2017; Bansal, 2018).

Heavy metals are among the most dangerous toxicants for human health. They are classified as thiol poisons that block sulfhydryl groups of proteins and disrupt metabolic processes in the body (at low doses), in large doses they can act as blockers and other functionally active groups of proteins - amine, carboxylic, etc (Godwill et al., 2019).

Soil is a unique natural formation in which the interaction of living and non-living matter is most intense, where biological and biogeochemical cycles of substances are formed.

Of the many indicators of the biological activity of the soil, soil enzymes are of great importance. Their diversity and richness make it possible to carry out successive biochemical transformations of organic residues entering the soil.

In terms of enzymatic diversity and enzymatic pool, the soil is the most diverse system. The transformation of animal and plant residues into humic substances is a complex biochemical process that takes place with the participation of extracellular enzymes immobilized by the soil, as well as various groups of microorganisms. A direct relation between enzymatic activity and the intensity of humification is shown (Hagmann et al., 2015; Kouchou et al., 2017).

The enzymatic activity is influenced by a number of natural factors - the chemical and physical composition of the soil, moisture, acidity (pH), temperature, etc. However, in recent years, due to the growth of anthropogenic load on soil, anthropogenic factors have an increasingly intense effect on enzymatic activity (Aiju et al., 2013, Nwaogu et al., 2014; Naimi, 2018).

The research of monitoring and diagnostics of soil cover by biochemical methods, in particular, indicators of the enzymatic activity of soil, show maximum efficiency. This diagnostic indicator provides high sensitivity, ease of determination and, low experimental error (Hagmann et al., 2015.)

Hydrolases are widespread in soil and play an important role in enriching them with nutrients that are mobile and sufficient for plants and microorganisms, destroying high molecular weight organic compounds. This class includes the following enzymes: urease, invertase, phosphatase, etc., whose activity is an important indicator of the biological activity of soil and is widely used to assess anthropogenic impact (Sharma et al., 2019).

Urease is an enzyme involved in the regulation of nitrogen metabolism in the soil, catalyzing the hydrolysis of urea to ammonia and carbon dioxide, causing the hydrolytic cleavage of the bond between nitrogen and carbon in the molecules of organic substances. Urease is found in all soils, and its activity correlates with the activity of all the main enzymes of nitrogen metabolism. The rate of hydrolysis of urea in the soil is influenced by temperature and soil acidity. Heavy metal salts, as well as aliphatic amines, dehydrophenols, and quinones, significantly inhibit urease.

In soil in the form of organic compounds, there is a large amount of phosphorus, coming with dying remains of plants, animals and microorganisms. The release of phosphoric acid from these compounds is carried out by a relatively narrow group of microorganisms with specific phosphatase enzymes. Phosphatase activity of the soil is determined by its genetic characteristics, physicochemical properties and the level of farming culture (Micuti et al., 2017).

Invertase catalyzes the hydrolytic cleavage of sucrose into equimolecular amounts of glucose and fructose, also affects other carbohydrates to form fructose molecules - an energy product for the life of microorganisms and catalyzes fructose transferase

reactions. The research by many authors has shown that the invertase activity reflects well the level of fertility and biological activity of soil (Maddela et al., 2017).

Enzymes belonging to the class of oxidoreductases catalyzes redox reactions that play a leading role in biochemical processes in the cells of living organisms, as well as in the soil. The most common oxidoreductases in soil are catalase and dehydrogenases, the activity of which is an important indicator of the genesis of soil.

The research by various authors (Mikanová et al., 2001; Makádi et al., 2019) has established that the activity of soil enzymes can serve as an additional diagnostic indicator of soil fertility and its changes as a result of anthropogenic impact. The use of enzymatic activity as a diagnostic indicator is facilitated by a low experimental error and a high stability of enzymes during storage of samples.

Thus, in the conditions of technogenic soil pollution, the search for measures to reduce the negative impact on the biosphere. One of the effective methods of reducing the negative consequences of soil contamination with heavy metals is the development of optimal systems for the use of fertilizers.

The purpose of this research was to study the effect of complex pollution by heavy metals on the enzymatic activity of podzolized chernozem under conditions of its sanitation.

MATERIALS AND METHODS

Developing agrochemical methods for decomposition of contaminated soil, in a stationary lysimetric experiment, a preliminary stage was carried out, in which the total content of Zn, Cu, Pb, Cd in podzolized chernozem, its hydrolytic acidity (pHhyd) in each lysimeter was studied and acidity was neutralized.

The soil at the experimental site has the following characteristics: pH_{KCl} 6.2; humus content - 3.2%, P_2O_5 - 229 mg kg^{-1}, K_2O - 250 mg kg^{-1} of soil. The studies were carried out on the Meshchera ecopolygon (Polkovo, Ryazan district), in four-fold repetition.

According to the regional gradation of soil pollution levels, compiled on the basis of the geochemical background, the content of Cu of 90 mg kg^{-1}, Zn - 110 mg kg^{-1}, Pb - 40 mg kg^{-1}, Cd - 0.6 mg kg^{-1} in the soil represent increased pollution.

Modeling of the increased complex level of soil contamination was carried out by adding to the soil. In this case, chemically pure salts were used: $Zn(CH_3COO)_2 \cdot 2H_2O$; $CuSO_4 \cdot 5H_2O$; $Pb(CH_3COO)_2$; $CdSO_4$.

For this, a soil layer of 20 cm depth was selected from the lysimeter. The calculated dose of salts of Cu, Zn, Pb, and Cd was thoroughly mixed with this soil and placed in the same lysimeter.

The effect of the following fertilization systems was studied: organic (cattle manure), organo-mineral and mineral, where double superphosphate was used periodically and annually in increased doses. For podzolized heavy loamy chernozem, the manure rate is 100 t ha^{-1} (Table 1). The norms of mineral fertilizers, depending on the crop, are adopted according to the recommendations for our zone (Ivanov & Derzhavin, 2008).

The calculated fertilizer rates were evenly and manually distributed over the surface of the lysimeter, and then the soil was dug to a depth of 12–15 cm. The soil surface was levelled. A mixture of herbs (fescue, timothy, clover) was sown as an equalizing crop.

As mineral fertilizers were used the urea (N - 46%), double superphosphate (P_2O_5 - 44%), potassium sulphate (K_2O - 48%).

Table 1. The scheme of carrying out the field lysimetric experiment

No of variants	Variants	Abbreviations in variant name tables
1	Control (without heavy metals and detoxification agents)	Control
2	Soil contaminated by a complex of heavy metals	Pollutants
3	Periodic application Cattle manure 100 t ha^{-1}	C100
4	Periodic application Cattle manure 100 t ha^{-1} + N60(N90)P60K60(K120) – annually depending on the crop (kg ha^{-1})	C100N1P1K1
5	P2 - periodic application of phosphorus, once every 2 years at a dose of 120 kg ha^{-1}, annual use N60(N90)K60(K120)	P2N1K1
6	P4 - periodic application of phosphorus, once every 4 years at a dose of 240 kg ha^{-1}, annual use N60(N90)K60(K120)	P4N1K1
7	P2 (e) - annual application of an increased dose of phosphorus (120 kg ha^{-1}) and optimal doses N60(N90)K60(K120)	P2(e)N1K1

The collection and analysis of soil samples for enzyme activity was carried out by methods generally accepted in enzymology and soil science. For comparative characteristics of different types of soils in terms of enzymatic activity, samples were taken at the end of the growing season (in september), in order to exclude the influence on the enzymatic activity of plants. An average soil sample from the top layer with a thickness of 0–5 cm was taken from each site. Uncontaminated soil without the use of fertilizers, was served as a control. The activity of catalase was determined by the gasometric method by the volume of released oxygen, based on measuring the rate of decomposition of hydrogen peroxide during its interaction with soil (Devyatova & Kramareva, 2008.)

To determine the dehydrogenase activity, colorless tetrazolium salts (2,3,5-triphenyltetrazolium chloride (TTC), which are reduced to red formazan compounds (triphenylformazan (TPF)), were used as a hydrogen acceptor. Determination of invertase activity was carried out by photocolorimetric method, which is based on taking into account the reducing sugars formed during the breakdown of sucrose (Khaziev, 2005). Phosphatase and urease activity was assessed using the Gaponyuk's and Malakhov's scale (Zvyagintsev, 1978) in mg P_20_5 10 g 1 h^{-1} and in mg NH_4^+ 10 g 24 h^{-1}, respectively.

The statistical processing of the results obtained was carried out by conventional methods (assessment of significance according to Fisher's and Student's criteria) using the Microsoft Excel software package.

RESULTS AND DISCUSSION

Effect of detoxification on hydrolytic enzymes of podzolized chernozem

Urease is one of the enzymes for the transformation of nitrogen compounds in the medium (Bansal, 2018). We noted a significant change in the urease activity of the soil against the background of its contamination by heavy metals and the use of detoxification (Table 2). In the control variant, the urease activity was 7.6 μg NH_4 10 g^{-1}

soil. In a soil sample with complex pollution, a sharp decrease in the activity of urease was observed - to 1.5 μg NH_4^+ g^{-1} of soil, that is less than the control by 80.26% (Table 3). This is due to the strong inhibitory effect of pollutants on urease. A decrease in urease activity to a minimum level indicates a weakening of the biochemical processes of the exchange of nitrogen-containing compounds, which may be the result of inhibition of the vital processes of microorganisms that synthesize urease.

According to the scale for assessing the degree of soil enrichment with enzymes (Table 4), this variant is characterized as very poor.

The application of various fertilizers contributed to the restoration of urease activity. However, it should be noted that the most effective method, according to our research, is an organo-mineral complex of fertilizers, the introduction of which made it possible not only to stop the negative effects of heavy metals, but also to increase the urease activity of the soil by 3.38 times (Table 2), i.e. it was 25.7 μg NH_4 10 g^{-1} of soil - medium enriched with this enzyme.

Table 2. Hydrolytic enzymes of podzolized chernozem contaminated by heavy metals using sanitation methods

Variants	Urease, mg NH_4 per 10 g for 24 h	Invertase, mg of glucose per 11 g for 24 h	Phosphatase mg P_2O_5 per 10 g for 1 h
Control	7.6	19.1	3.3
Pollutants	1.5	9.3	0.6
C100	18.1	47.1	4.1
C100N1P1K1	**25.7**	**51.2**	**4.9**
P2N1K1	8.7	26.3	3.2
P4N1K1	9.2	28.0	3.1
P2(e)N1K1	8.9	25.2	3.0
$LSD_{0.95}$	0.04	0.04	0.03

Table 3. Changes in enzyme activity in podzolized chernozem (in % compared to control)

Variants	Urease	Invertase	Phosphatase	Catalase	Dehydrogenase
Pollutants	-80.26	-53.03	-81.81	-50.00	-85.71
C100	+144.73	+36.87	+24.24	+19.44	+20.00
C100N1P1K1	+238.16	+158.59	+48.48	+55.56	+45.71
P2N1K1	+14.47	+32.83	-03.03	-8.33	-11.73
P4N1K1	+21.05	+41.41	-06.06	-11.11	-8.57
P2(e)N1K1	+17.1	+27.27	+27.27	-11.11	-11.43

The invertase activity of the control variant of the experiment is characterized as average - 19.1 mg of glucose per 1 g for 24 h. Especially strong depression of invertase is observed in the variant without the introduction of sanitation means, where its decrease in comparison with the control variant was by 53.03%. The use of fertilizers has led to a decrease in the negative impact on the soil of complex pollution by heavy metals. The best effect was obtained when using C100N1P1K1, the invertase activity here was 47.1 mg of glucose per 1 g for 24 h, which is higher by 158.59% in comparison with the control variant of the experiment. Probably, the enzymes show a higher resistance due to the organomineral complex, which stabilizes them in the soil environment.

The total phosphatase activity of the soil depends on the content of humus and organic phosphorus, which is a substrate for the enzyme. Soil contamination led to a decrease in phosphatase activity in comparison with the control variant by 81.81% and amounted to 0.6 mg P_2O_5 per 10 g per 1 hour, which characterizes the chernozem as very

poor (Table 3) in terms of the degree of enrichment with this enzyme. When using detoxification, an increase in phosphatase activity occurred from 3.0 (variant P2 (e) N1K1) to 4.9 mg P_2O_5 (variant D100N1P1K1) per 10 g per 1 h.

Table 4. Scale for assessing the degree of soil enrichment with enzymes according to D.G. Zvyagintsev (Zvyagintsev, 1978)

Soil enrichment rate	Catalase, O_2 cm^3 g^{-1} for 1 min	Dehydrogenase, mg TPF per 10 g for 24 hours	Invertase, mg of glucose per 1 g for 24 h	Urease, mg NH_4, per 10 g for 24 h	Phosphatase, mg P_2O_5 per 10 g per 1 h
Very poor	< 1	< 1	< 5	< 3	< 0.5
Poor	1–3	1–3	5–15	3–10	0.5–1.5
Average	3–10	3–10	15–50	10–30	1.5–5.0
Rich	10–30	10–30	50–150	30–100	5–15
Very rich	> 30	> 30	> 150	> 100	> 15

The influence of chemical compounds in the composition of fertilizers on the enzymatic activity can be either direct (inhibitors or activators of the action of enzymes), or indirect (influence on the growth and development of soil organisms and plants that produce enzymes). A number of authors (Khaziev, 1982; Gianfreda et al., 2005) say that, in general, fertilizers have a powerful effect on the enzymatic activity of soils. On all soils, complex mineral fertilizer (NPK) was more effective, especially when combined with organic fertilizers, as opposed to individual types. At the same time, the effect of mineral fertilizers on various enzymes is not the same (Burns et al., 2013).

High doses of phosphorous fertilizers reduced the activity of phosphohydrolytic enzymes, and at low doses their activity increased. For nitrogen fertilizers, the activity of all enzymes, especially phosphatase and invertase, increases (Khaziev, 2018). Stable high enzymatic activity is achieved by systematic application of fertilizers to the soil, especially organic fertilizers together with mineral fertilizers, which corresponds to our research.

The effect of detoxification on oxidoreductases in podzolized chernozem

Catalase plays an important role in the neutralization of hydrogen peroxide, toxic to soil living organisms, which enters the soil as a result of their high physiological activity during unfavorable living conditions. Researchers have noted the sensitivity of this enzyme to the content of heavy metals (Murali & Patel, 2017; Jaworska & Lemanowicz, 2019; Aponte et al., 2020).

Soil contamination by a complex of heavy metals led to a 2-fold decrease in catalase activity compared with the control variant of the experiment and amounted to 1.8, O_2 cm^3 g^{-1} per 1 min, which characterizes it as poor in the degree of enrichment with this enzyme (Table 5).

Table 5. Oxidoreductases of podzolized chernozem contaminated by heavy metals using sanitation methods

Variants	Catalase, O_2 cm^3 g^{-1} per 1 min	Dehydrogenase, mg TPF per 10 g for 24 h
Control	3.6	3.5
Pollutants	1.8	0.5
C100	4.3	4.2
C100N1P1K1	5.6	5.1
P2N1K1	3.3	3.1
P4N1K1	3.2	3.2
P2(e)N1K1	3.2	3.1
$LSD_{0.95}$	0.04	0.03

The use of mineral fertilizers promoted the activation of catalase, however, it did not fully compensate for their negative effect and was 8.33–11.11% lower than in the control variant. The use of C100N1P1K1 led to an increase in catalase activity by 55.56% and amounted to 5.6 O_2 cm^3 g^{-1} per 1 min, which classifies the soil as a medium supplied with this enzyme (Table 4).

Dehydrogenases are enzymes that are involved in the respiration process, splitting off hydrogen from oxidized substrates. The activity of dehydrogenases is an informative indicator, since it depends on the intensity of the processes of nitrification, nitrogen fixation, respiration, and oxygen absorption by the soil. Therefore, even with a low level of technogenic load on the soil, its dehydrogenase activity decreases. This makes it possible to use indicators of the activity of these enzymes in the diagnosis of soil pollution by heavy metals (Wiatrowska et al., 2015, Łukowski & Dec, 2018)

Soil microorganisms have different resistance to heavy metals and are in constant interaction with each other and the soil. Therefore, the reaction of the microbiocenosis to heavy metals is determined by their interaction with the soil, their effect on microorganisms and on the competitive relationships of microorganisms (Micuti et al., 2017).

In general, these processes, according to Wolińska & Stepniewska (2012), are reflected in the change in the level of dehydrogenase activity, that is, in its inhibition, depending on the specific ecotoxicological situation. Explaining the reasons for the decrease in the enzymatic activity of the soil (Burns et al., 2013, Khaziev, 2018) under the influence of heavy metals, they are attributed to blocking the respiration chains and delaying the synthesis of enzymes of microorganisms while suppressing their growth.

According to the obtained data, presented in the Table 5, the activity of dehydrogenases in the variant 'Pollutant' decreased by 7 times compared to the control and amounted to 0.5 mg TPF per 10 g per 24 hours, which characterizes chernozem as very poor in the degree of enrichment with this enzyme. The use of mineral fertilizers made it possible to reduce the toxic effect of heavy metals, however, these sanitation measures did not allow to return to the indicators of clean, uncontaminated soil. The organo-mineral complex of fertilizers had the best effect. In this variant, there was an increase in dehydrogenase activity by 45.71% in comparison with the control variant.

Adding fertilizers to the soil not only improves the nutrition of plants, but also changes the conditions for the existence of soil microorganisms, which also need mineral elements. Under favorable climatic conditions, the number of microorganisms and their activity after fertilizing the soil increases significantly (Godwill et al., 2019).

CONCLUSIONS

In conditions of soil contamination with heavy metals, it is important to find ways to overcome their negative impact on agrocenoses. One of the methods can be the means of agrochemistry-organic and mineral fertilizers. The use of detoxicants helps to reduce the negative effect of toxicants on the studied indicators of the enzymatic activity of the soil. In the conducted studies, it was revealed that the organomineral system of fertilizers had the greatest protective effect on the enzymatic activity, which allowed to increase the activity of soil urease by 3.38 times, invertase - by 2.47 times, phosphatase - by 1.48 times, dehydrogenase - by 1.46 times, catalase - by 1.60 times.

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