

Comparison of Natural Abiotic Factors and Pollution Influence on the Soil Enzymatic Activity

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ABSTRACT

The enzymatic pool of the soil is formed over a long period of time under the influence of many natural factors. Therefore, the information on the lability of soil enzymes under the influence of the main abiotic factors is necessary for the diagnosis of the anthropogenic influence on ecosystems. The aim of the study was to determine the variability of the activity of catalase, invertase, and urease in sod-podzolic and grey forest soils in response to the main abiotic factors and model pollution. The studied areas belonged to the subzones of middle and southern taiga and mixed forests, the average annual temperature ranged from 2.9 to 6.6 °C, and the pH_{KCl} level of extracts from soils was in the range of 4.2–5.6. It was shown that the activity of exozymes in the soil of southern taiga and mixed forests depends most of all on the average annual temperature and pH level. No relationship between the enzyme activity and soil organic carbon was found. Artificial soil salinity led to an increase in the activity of invertase and urease. Soil contamination with zinc (25.5 and 255.0 mg/kg of dry soil) suppressed only the invertase activity. Thus, the influence of natural abiotic factors on the activity of soil enzymes was more often found than the chemical load.

Keywords: soil, soil enzymes, abiotic factors, average annual temperature, soil pH, pollution, zinc, salinity.

INTRODUCTION

The activity of soil enzymes is an important parameter for assessing the ecological state of the soil (Kolesnikov et al. 2019). The activity of soil enzymes is known to change as a result of soil contamination with a variety of chemicals (Igalavithana et al. 2017; Datt and Singh 2019). At the same time, the authors noted a significant lability of the activity of soil enzymes. As a result, the data on the relationship between pollution level and the enzyme activity is difficult to interpret (Bünemann et al. 2018).

For environmental studies, it is recommended to determine the activity of a complex of enzymes, for example, from oxidases – catalase, from carbohydrases – invertase, and from amidases – urease (Amat et al. 2021). On the one hand, this approach gives a more complete picture of the effect of the toxicant on subsurface processes, and on the other hand, it also complicates the interpretation

of the results. For example, seasonal variability of individual soil enzymes in soil under olive trees was shown to range from 29 to 71% with no consistent temporal trend (Garcia-Ruiz et al. 2009). Even a change in the species composition of plants within the same agricultural field changes the enzymatic activity. The activity of protease, sucrase, and acid phosphatase was higher in intercropping of peanut crops with tea plants, while the activity of urease and catalase was higher in a peanut monoculture (Farooq et al. 2018).

In this regard, the study of the reasons for the variability of the activity of soil enzymes and other functions of soil biota is an urgent task, the solution of which is aimed at an objective diagnosis of the ecological state of soils. Soil type and temperature fluctuations are important for activation of soil enzymes and further bioavailability of copper (Fu et al. 2018). The linear relationship between soil respiration and water content was proved (Cook and Orchard 2008). The presented data suggests that

the “enzyme activity-factor” dependence needs to be clarified for the specific studied area. Such data will become a foundation for monitoring changes in the enzyme activity in selected areas during subsequent anthropogenic impacts.

The aim of this work is to determine the variability of the activity of catalase, invertase and urease in sod-podzolic and grey forest soils in response to the main abiotic factors, i.e. average annual temperature, pH, and soil organic matter content, as well as to compare these dependencies with the effects of artificial soil salinity and pollution (using the example of zinc).

MATERIAL AND METHODS

The soil samples were taken by using the “envelope method” in July–August 2015–2016, dried to the air-dry state without heating, and homogenized. The studies included establishing the action of certain edaphic and chemical factors that could potentially affect the level of the enzymatic activity of soils. For this purpose, the content of organic carbon (SOC) was determined with the spectrophotometric method; pH of salt extract from soil samples (pH_{KCl}) – by using the potentiometric method; soil salinity – through the electrical conductivity of water extracts from soils by the conductometric method; the content of mobile forms of zinc – via atomic absorption spectroscopy using the SPEKTR-5-4 (Russia) device with preliminary extraction of soil samples with an acetate-ammonium buffer ($\text{pH} = 4.8$)

(State standard 26213-91 1991; State standard 26483-85 1985; State standard 26423-85 2011; Kuznetsov et al. 1992). The characteristics of the samples are presented in Table 1.

The contribution of soil contamination with zinc to the variability of the enzymatic activity was shown in the supplementary model experiment. In chemically inert vessels, the soil was cultivated within 14 days with an additional chemical load – of 25.5 mg and 255.0 mg of zinc per 1 kg of dry soil (in terms of a chemical element). Model toxicant used involved zinc EDTA (ethylenediaminetetraacetic acid) – Zn 15%. Soil salinity was simulated in a similar way. Excess doses (5 g/kg) of mineral fertilizer N P K S 24:6:6:2 were applied to the soil. The soil was moistened to its natural state (moisture content 60%). The soil without additives was the control one.

Next, the level of the enzymatic activity of the soil was determined. The activity of catalase was determined by using the gasometric method according to A. Sh. Galstyan, taking into account the amount of released molecular oxygen. The invertase and urease activities were measured by means of the colorimetric method, taking into account the amount of reducing hexoses formed during the hydrolysis of sucrose and the amount of ammonium ions with Nessler’s reagent, respectively (Workshop on agrochemistry, 2001). The enzymatic activity of the soils was assessed using the Gaponyuk-Malakhov scale (Table 2).

The results were processed statistically with the calculation of the arithmetic mean, standard

Table 1. The studied soil samples and their characteristics

Number of the sample	Region of the Russia	Soil type / natural area	Average annual temperature, °C (rp5.ru)	pH_{KCl}	SOC
1	The Komi Republic	Sod-podzolic / taiga	+2.9	4.2±0.1	1.6±0.3
2	The Kirov Region	Sod-podzolic / taiga	+4.1	5.5±0.1	1.3±0.2
3	The Mari El Republic	Sod-podzolic / mixed forests	+5.6	5.4±0.1	2.1±0.4
4	The Nizhni Novgorod Region	Grey forest / mixed forests	+5.8	5.4±0.1	1.4±0.3
5	The Chuvash Republic	Grey forest / mixed forests	+6.6	5.6±0.1	5.6±0.6

Table 2. Scale for comparative assessment of the soil biological activity (Gaponyuk and Malakhov 1985)

The biological activity of the soil	Catalase, $\text{sm}^3 \text{O}_2 \text{g}^{-1} \cdot \text{min}^{-1}$	Invertase, $\text{mg C}_6\text{H}_{12}\text{O}_6 \text{g}^{-1} \cdot \text{day}^{-1}$	Urease, $\text{mg N-NH}_3 \text{10 g}^{-1} \cdot \text{day}^{-1}$
Very weak	<1	<5	<3
Weak	1–3	5–15	3–10
Average	3–10	15–50	10–30
High	10–30	50–150	30–100
Very high	>30	>150	>100

deviation, significant difference between the compared samples (according to Student's t-test), the analysis was carried out with the calculation of the Pearson correlation coefficient (r), as well as the analysis of variance with the calculation of Fisher's test (F).

RESULTS AND DISCUSSION

The enzyme activity of different soil types and average annual air temperature

The environmental temperature is one of the main factors that determine the species and quantitative composition of the soil microorganisms. In turn, the reactions catalyzed by exoenzymes of microorganisms obey the Le Chatelier principle, in which a change in temperature leads to a shift in the equilibrium of the chemical system. Therefore, first of all, the dependence of the enzyme activity on the average annual temperature of the area of sampling was analyzed (Fig. 1).

According to the scale of comparative assessment of the biological activity of the soil, the studied soil samples (Table 2) were poorly supplied

with catalase, moderately and highly supplied with invertase, as well as moderately and very highly supplied with urease (Fig. 1 a, b, c).

In order to differentiate the influence of edaphic factors on the enzymatic activity of soils, the analysis of variance was carried out. The highest level of the enzymatic activity was found in the soil samples taken at sites geographically located to the south and characterized by higher mean annual air temperatures. The effect of the temperature factor on the activity level of invertase ($F = 6.3$; $p = 0.0194$) and urease ($F = 7.87$; $p = 0.0106$) was more significant than the effect of the soil type ($F = 0.08$; $p = 0.7775$ and $F = 1.23$; $p = 0.2956$, respectively). It was revealed that in sod-podzolic soils (plots No. 1, 2, 3), the activity of enzymes varied in a wider range than in grey forest soils (plots No. 4, 5).

The obtained data are consistent with the results of other authors. Thus, in the work (Eliseeva, 2018), the catalase activity in the soils of the southern territories was higher than in the northern ones.

The significant dependence of the enzymatic activity on the average annual temperature suggests that the comparison of soils in the territories with different heat supply is not correct, even if the soils are of the same type.

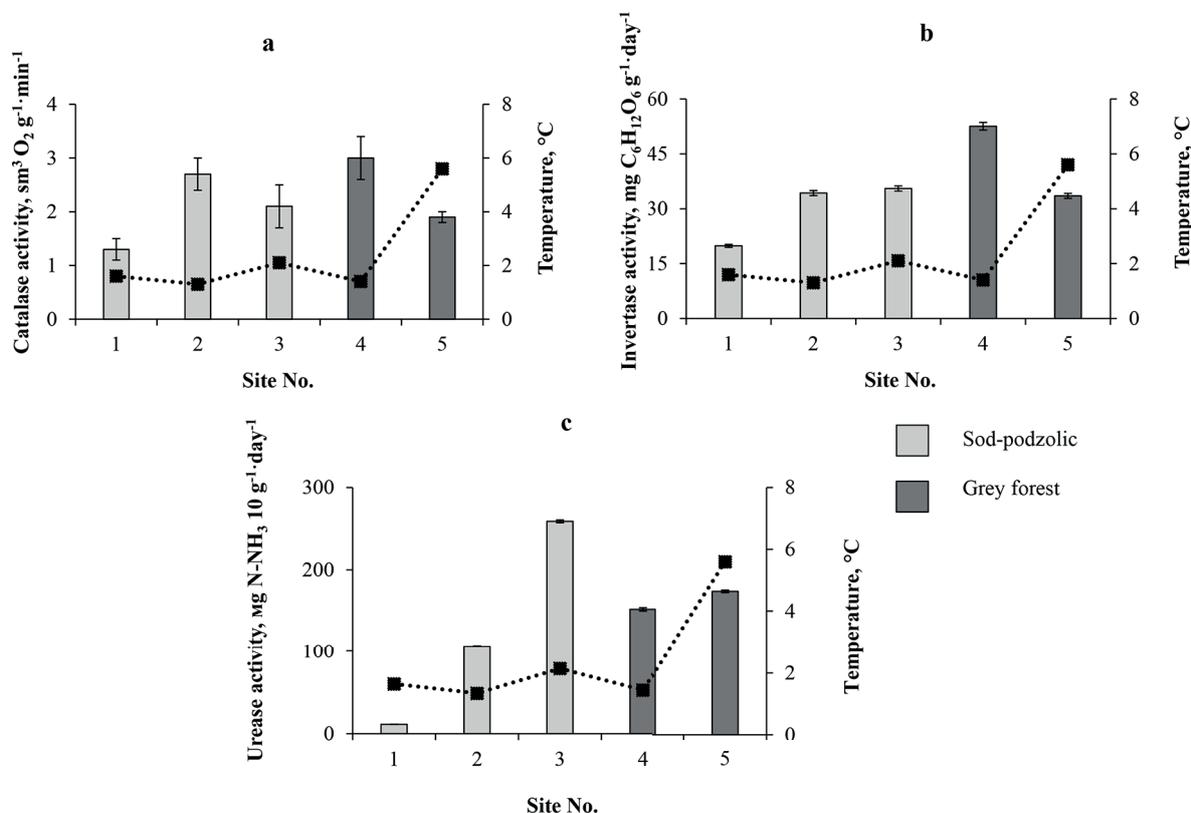


Figure 1. Catalase (a), invertase (b) and urease (c) activity of soil samples taken under different temperature conditions

The enzyme activity and organic matter content in soil

No dependence between the level of the enzyme activity and the organic matter content in soil was found. The correlation coefficients were mathematically insignificant: for the values “the catalase activity – SOC” $r = -0.32$; for “the invertase activity – SOC” $r = -0.11$; for “the urease activity – SOC” $r = 0.30$. Perhaps this is due to the fact that the soils are very low (1–2); low (2–4) and medium humus (4–6% org.) (No. 1, 2, 4; 3 and 5, respectively). This fact can also be explained by the regularity shown in the work (Hsiao et al. 2018): the penetration of microbes into the soil is limited by phosphorus and nitrogen, not by carbon. However, in the soils containing higher (more significant) concentrations of organic matter, such relationships were identified. For example, in the soils of China (Shandong Province), the activities of urease, alkaline phosphatase, and catalase were significantly positively correlated with the soil organic matter (Liu et al. 2017).

It is known that the pH of the environment has a significant effect on the redox processes occurring in soils, including with the participation of enzymes. At the same time, for the action of each enzyme an optimal pH value of the medium, generally close to a neutral and slightly acidic reaction, is required (Khaziev, 2018).

The soil pH level was judged by the pH values of salt extracts from the analyzed samples. The obtained soil pH values in plots No. 1–5 varied from 4.2 (very strongly acidic) to 5.6 (close to neutral according to the Kornilov scale, 1947). At the same time, the presence of a close correlation between the values of this parameter and the level of the enzymatic activity of soils in taiga and mixed forests was noted. In the case of the catalase activity, the correlation coefficient (r) was 0.69; the invertase – $r = 0.67$; the urease – $r = 0.76$. In contrast to the catalase and invertase, the effect of the acidity of the soil solution on the urease activity was more pronounced. In the soils with the pH close to neutral, the level of the

urease activity was significantly higher than in a more acidic environment.

For other types of soils and natural zones, the authors obtain similar results. It was shown in (Wang et al. 2006) that a drop in pH significantly decreased the activity of the soil alkaline phosphatase, arylsulfatase activity, nitrification potential, and respiration. However, the activity of the acid phosphatase increased with decreasing pH.

Thus, in sod-podzolic and gray forest soils of taiga and mixed forests, characterized by the pH close to neutral, the enzyme activity was higher than in more acidic soils. Among the studied enzymes the urease was found to be the most sensitive to soil acidity.

The enzyme activity and soil salinity

The total content of salts in the soil solution was judged by the electrical conductivity of the water extract from the studied samples (1:5). A sample of sod-podzolic soil was selected from the territory of the southern taiga subzone and the natural salt content of the soil solution and the activity of three soil enzymes were determined. Then, artificial soil salinity was carried out under the conditions of a model experiment (see “Materials and Methods”). The influence of the degree of soil salinity on the enzymatic activity is shown in Table 3.

Despite the fact that the salinity level of the soil samples differed by two orders of magnitude, no significant differences in the level of the catalase and invertase activity were found. For urease, an increase in its activity was recorded with an increase in salinity ($p < 0.05$), which is possibly associated with an increase in the concentration of nitrogen-containing compounds in the soil. In general, these data are consistent with the results of other authors. For example, in (Shahariar et al. 2021) it was shown that the effect of groundwater salinity on the activity of extracellular soil enzymes was non-significant ($p > 0.05$).

Thus, according to the degree of resistance to salinity, the enzymes are arranged in the following order: catalase > invertase > urease. An artificial increase in the salt content in the soil led to

Table 3. Dependence of the enzymatic activity of the soil on the salt content

Conductivity, $\mu\text{S}/\text{sm}$	Enzyme activity		
	Catalase, $\text{sm}^3 \text{O}_2 \text{g}^{-1} \cdot \text{min}^{-1}$	Invertase, $\text{mg C}_6\text{H}_{12}\text{O}_6 \text{g}^{-1} \cdot \text{day}^{-1}$	Urease, $\text{mg N-NH}_3 \text{10 g}^{-1} \cdot \text{day}^{-1}$
47.3	1.4 ± 0.1	18.8 ± 0.5	11.6 ± 0.1
1013.5	1.3 ± 0.1	19.9 ± 0.6	15.6 ± 0.3

an increase in the activity of invertase and urease. The found relationship between the activity of the soil enzymes and salinity requires further refinement in larger experiments.

The enzyme activity and soil contamination

Zinc was chosen as a chemical agent capable of blocking the activity of the soil enzymes. In most of the studied soil samples, no significant differences in the level of the catalase and urease activity were found under the action of 25.5 and 255.0 mg/kg of zinc (Fig. 2a, c). Both a decrease in the activity of these enzymes under the influence of a higher dose of zinc in the experiment, and its increase were noted. The provision of the soil with catalase and urease under the action of increasing doses of zinc did not change.

Compared to the catalase and urease, invertase was more sensitive to the action of zinc. For all studied samples, with the exception of the soil sampled at plot No. 3, a decrease in the level of the invertase activity with an increase in the dose of zinc in the soil was noted (Fig. 2b). Under the action of the lowest dose of zinc (25.5 mg/kg), the invertase activity

varied within the values typical for the “moderately supplied” category, and under the influence of an increased dose (255.0 mg/kg), the enzyme activity belonged to the category “poorly supplied”.

Opposite reactions of the enzymes were noted by scientists when other metals are exposed to the soil. For example, Pb had no effect on the activity of urease, acid phosphatase and dehydrogenase, but it stimulated the activity of alkaline phosphatase (Yang et al. 2007).

Thus, no correlation was found between the zinc content in the soil and the level of the enzyme activity, with the exception of invertase. For invertase, a decrease in the level of the activity was found in response to an increase in the concentration of zinc in the soil to 255.0 mg/kg. The findings are consistent with those of other studies (Baikhamurova et al. 2020).

CONCLUSIONS

The results of this work showed that abiotic factors affect the activity of soil enzymes, but not equally. The increased enzyme activity was

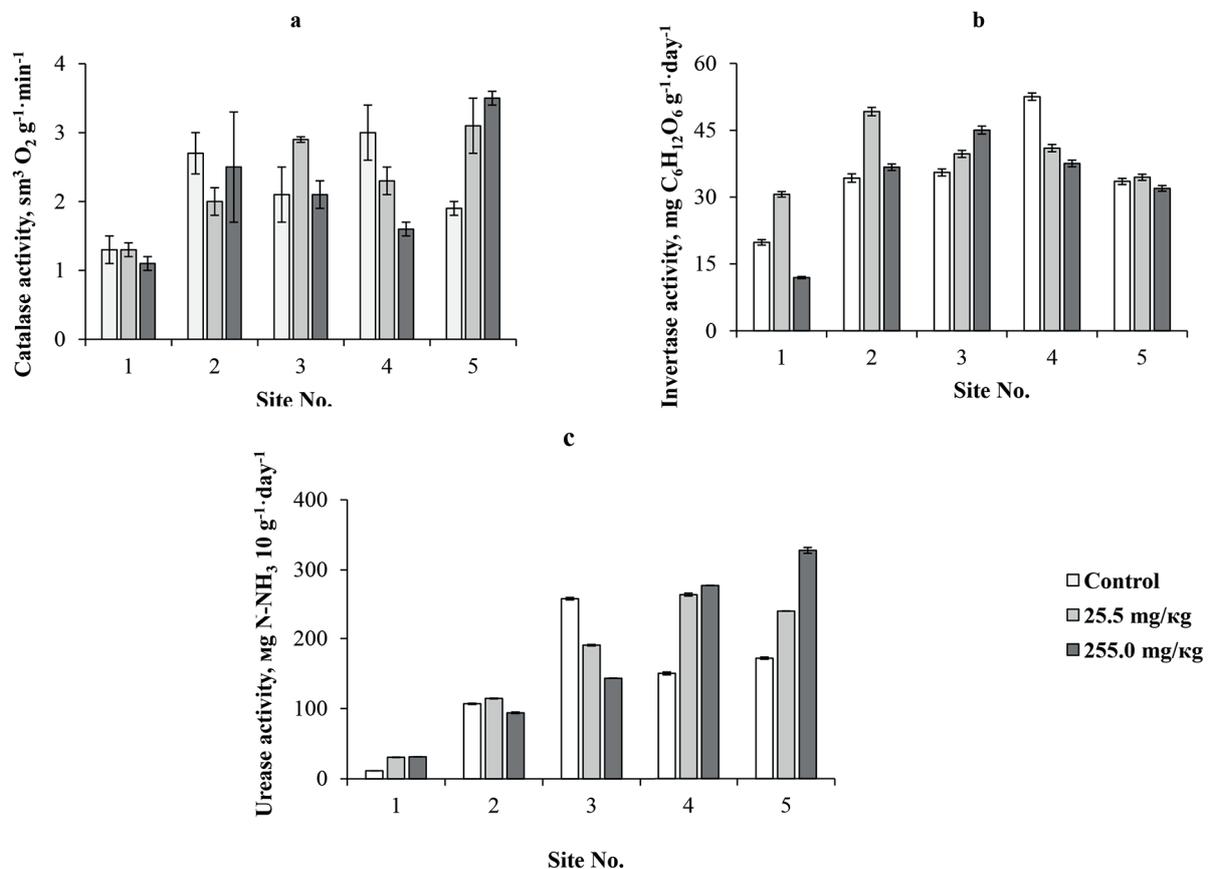


Figure 2. The effect of zinc on the catalase (a), the invertase (b) and the urease (c) soil activity

observed in the soils sampled in the areas with a higher heat supply, as well as in the soils with the pH level close to neutral. No relationship was found between the accumulation of organic matter in the soil and the activity of catalase, urease, and invertase.

Natural abiotic factors had a greater effect on catalase, urease, and invertase than artificial pollution (application of mineral fertilizers up to electrical conductivity of 1013.5 $\mu\text{S}/\text{sm}$ and zinc up to 225.0 mg/kg). Out of the studied enzymes, the most labile are the representatives of the class of hydrolases (invertase and urease). Catalase (a class of oxidoreductases) turned out to be the most resistant to the action of chemical factors. The totality of the data suggests that the indication of soil salinity can be carried out using urease, and zinc contamination using invertase.

The enzymatic activity, of course, depends on the presence of pollutants in the soil that affect microorganisms and their exoproducts – soil enzymes. However, these reactions depend on the combination of factors: the chemical nature of the toxicant, the dose of the toxicant, the form in which the toxicant is present in the soil solution, and the characteristics of the enzyme that is the recipient of the effect.

Thus, when choosing an informative indicator of soil pollution from among exoenzymes, it is necessary to compare the effect of natural abiotic factors and presumed pollution on soil enzymes. This approach will make it possible to select the most sensitive indicators and facilitate the explanation of the “contamination - the enzyme activity” relationship.

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