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ENZYMATIC ACTIVITY OF THE SOIL AS AN INDICATOR OF ENVIRONMENT CONTAMINATION

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S u m m a r y. Research on the enzymatic activity of the soil was located within the range of the influence of the „Puławy S.A" Nitrogen Plant (51^0 25' N; 21^0 57' E). The research points were selected on the Northeastern side (on the major wind rose) of the Plant and 50, 200, 400, 600, 1000, and 2000 m away from it. At the point 2000 m away from the Plant, the soil samples were taken in the habitat of fresh forest and on the adhering cultivation field from under winter wheat. The soil of the texture of the weakly loamy sand had incorrect exchange adsorption. During the period of the research, a decrease in the pH value of the soils along with the increasing distance from the Plant was observed. The lowest activity of dehydrogenase, phosphatase, and protease was found in the soils of the research point closest to the Plant (50 m), which was related to the strong soil environment contamination with heavy metals, especially zinc, which is extremely toxic to soil microorganisms and enzymes. Opposite tendencies were denoted in the case of urease. The activity of that enzyme was the greatest in the research points located closest to the Plant. It is worth to mention that the activity of all the investigated enzymes in the soil from the point 200 m away from the Plant was significantly higher, as compared to the enzymatic activity of the soils from the point 400 m away from the Plant. Within the research point 2000 m away from the Plant, the enzymatic activity in the forest soil was significantly lower than in the soil of the cultivation field.

K e y w o r d s : soil, enzymatic activity, pollutants.

INTRODUCTION

Long lasting influence of air pollutants on plant associations and soils deviated the ecological balance of the ecosystem, which was indicated by the loss of the environmental resistance, elimination of the processes of self-cleaning and biological degradation of the soil. Extremely unfavorable environmental conditions occurred in the areas located east of the emitter [17]. A clear decrease in the intensity of the emission from the Nitrogen Plant occurred in the beginning of the 90's [19]. The most recent research [3,4], however, did not prove any improvement in the state of the soil environment within the range of influence of the Nitrogen Plant.

MATERIALS AND METHODS

The research was located within the range of influence of the "Puławy" S.A. Nitrogen Plant (51°25' N; 21°57' E) on Dystric Cambisols derived from aeolian sands of various thickness overlaying fluvioglacial sands in the ground of the following profile: Ofh-AhE-BvBhfe-Bv-CBv-C-R. The area is located in eastern Poland on the right bank of the central Vistula River. The sands form rows of dune forms and a wide slightly undulating aeolian cover, placed from 120 to 130 m above the sea level [18]. The height of the dune reaches 10 m.

Study sites were located north-east (on the predominant wind rose) 50, 200, 400, 600, 1000, and 2000 m away from the Nitrogen Plant (NP). This area is characterized by a highly undulating dune relief and a lower ground water level. The soils show low water capacity, weak capability of water retention, and high air permeability [19].

Study sites located 50, 200, 400, and 600 m away from the emitter are placed on the denuded surfaces without organic cover, on which there are loose sands of the parent material slight features of pedogenic transformations. Sites 1000 and 2000 m away from the Plant, in the line of air polluted by the emission, are in the IIIrd zone of hazard to the forest in the habitat of fresh forest with soil covered by tussocks of verrucose birch (*Betula verrucosa* L.) wildings of various age, with a single American bird cherry (*Padus serotina* Ehrh.) and several single 60-80 year-old pine parent trees (*Pinus sylvestris* L.) left from the destroyed stand of pine-trees. In the site located 2000 m away from the Plant, the soil samples were taken also in the adhering cultivation field from under winter wheat. Complementary mineral fertilization under the winter wheat was as follows:

N-100; P - 80; K - 120 kg/ha. The fertilizers were applied once a year in the form of ammonium nitrate, 60% potassium salt, and triple superphosphate. In 1997, 1000 kg/ha of magnesium lime (32% CaO and 5.6% MgO) was used.

Soil samples for laboratory analysis were taken from particular study sites from the 0-20 cm layer in the second decade of September 2000. The content of Cd, Cu, Ni, Pb, and Zn was measured in those samples using the method of ICP emission spectrometry using the Leeman Labs PS 950 apparatus. Initial mineralization of the samples was carried out in Prolabo microwave ovens using the mixture of nitrogen acid and ultrachlorine acid (1:1, v/v). Measured was the total content of the investigated elements. The activity of the investigated enzymes was analyzed in the soil of natural humidity and the results were converted into absolutely dry soil mass each time measuring the humidity of the investigated soil. The activity of the following enzymes was measured: dehydrogenase [28]; phosphatase [27]; urease [30]; and protease [20]. The physical-chemical properties of the investigated soil were measured using the following methods [22]: soil pH in 1M KCl potentionmetrically; total of exchangeable bases (TEB) and total cation exchange capacity (CEC) - the Kappen method with calculation of the base saturation (BS); total organic carbon (TOC) - the Tiurin method; total nitrogen (TN) - the Kjeldahl method; nitrate nitrogen - colorymetrically, using the modified brucine method; ammonium nitrogen - colorymetrically, using the Nessler method.

RESULTS

The investigated soils had loose texture (83 - 87% content of the 1- 0.1 mm fractions; 9-12% content of the 0.1 - 0.02 mm fractions; 3-5% content of the <0.02 mm fractions) and defective sorptive properties: low CEC - 6.3 - 7.8 cmol(+) kg^{-1}; low total of TEB - 2.1-3.6 cmol(+) kg^{-1}; and low BS - 28-53% (Tab. 1).

A decrease in the value of the soil pH was denoted along with increasing distance from the Nitrogen Plant. In the study sites located the closest to the emitter (50 and 200 m) there was a significant deacidification of the soil. The greatest growth in the soil pH, up to 6.2 units of pH_{KCl}, was observed in the site located 200 m away from the Plant. The greatest acidification was stated in the forest soils, where the pH_{KCl} ranged from 2.4 to 2.7 (Tab. 1). In the study site 2000 m away from the emitter, the soil used agriculturally had a much lower level of acidification than the forest soil from the closest neighborhood. The pH of the soil from the adhering cultivable field was 1.9 units higher than that of the forest soil.

Table 1. Properties of the soils

Distance from NP (m)	pH_{H2O}	pH_{KCL}	TEB (cmol(+) kg^{-1})	CEC (cmol(+) kg^{-1})	BS (%)
50	6.3	5.2	2.7	6.4	42
200	7.3	6.2	3.6	6.8	53
400	5.8	4.9	2.5	6.3	39
600	3.8	2.7	2.1	6.7	31
1000	3.7	2.5	2.4	7.5	32
2000 – forest soil	3.6	2.4	2.2	7.8	28
2000 – arable soil	5.5	4.3	2.8	6.4	43

The content of humus in the soils, presented in Tab. 2, in the form of TOC was low, with C-organic from 0.56 to 1.16 g/kg in particular study sites. Also the content of total nitrogen in the soils was low - from 0.03 to 0.09 g/kg (Tab. 2). A significant decrease in the content of those elements in the investigated soils was observed in the sites that lacked natural plant cover and were located closest to the Plant.

The value of the TOC:TN ratio ranged from 12.4 in the cultivated soil to 18.6 in the site located closest to the Plant (Tab. 2).

Small content of the mineral forms of nitrogen $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ was stated in the investigated soils. The amount of those components was from 41.6 to 54.3 mg/kg of soil for the ammonium form and from 5.9 to 70.2 mg/kg of soil for the nitrate form (Tab. 2). In the mineral nitrogen in the soils, the content of the ammonium form was usually several times higher than the nitrate form. Only in the site located 200 m away from the emitter, where the soil had the highest pH, the NH_4^+: NO_3^- ratio had the value <1 (Tab. 2).

In the soil of the study sites located 50, 200, 400, and 600 m away from the emitter, in the surfaces that lacked organic cover, the concentration of Cd, Cu, Ni, Pb, and Zn was several times higher than the content that is considered a natural background [16]. The content of those elements in the soil decreased significantly with increasing distance from the Nitrogen Plant. The soil in the site 600 m from the Plant had a several times lower content of Cd, Cu, Ni, Pb, and Zn (6, 3, 2, 4, and 3 times - respectively) than the soil in the site located closest to the emitter.

The soil in the sites 1000 and 2000 m away from the Plant had a natural content of the investigated heavy metals (Tab. 3).

Table 2. Total organic carbon (TOC) and nitrogen (TN, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$) in the soils

Distance From NP (m)	TOC (g kg^{-1})	TN	TOC:TN	$N\text{-}NH_4^+$ (mg kg^{-1})	$N\text{-}NO_3^-$	NH_4^+: NO_3^-
50	0.56	0.03	18.6	46.2	29.1	1.6
200	0.91	0.06	15.1	48.5	70.2	0.7
400	0.72	0.04	18.0	41.6	19.8	2.1
600	0.76	0.05	15.2	54.3	6.3	8.6
1000	1.01	0.06	16.8	42.8	9.6	4.4
2000 – forest soil	1.16	0.07	16.5	47.9	5.9	8.1
2000 – arable soil	1.12	0.09	12.4	45.1	46.5	0.9
$LSD_{0.05}$	0.2	0.02	0.4	0.8	0.3	

Table 3. Content of the selected heavy metals in the soils

Distance From NP (m)	Cd	Cu	Ni (mg kg^{-1})	Pb	Zn
50	4.3	37.2	21.8	167.0	353.0
200	3.0	26.1	16.3	117.9	148.1
400	1.2	15.6	11.2	75.0	108.6
600	0.7	11.8	9.6	37.3	96.0
1000	0.3	11.2	8.8	24.2	54.0
2000 – forest soil	0.2	8.7	8.2	22.1	52.0
2000 – arable soil	0.4	10.4	13.7	24.7	57.0
$LSD_{0.05}$	0.1	1.2	3.2	1.4	7.7

Table 4 contains data of the enzymatic activity of the soil in the study sites. The greatest enzymatic activity was found in the soil of the cultivable field. The activity of dehydrogenase, phosphatase, and protease in the site located closest to the Plant was the lowest. Soil in that site, comparing to the soil from the cultivable field had statistically importantly twice as low activity of dehydrogenase, and three times as low activity of phosphatase and protease. The activity of urease in the sites located closest to the emitter (50 and 200 m) was about the same as in the cultivable field and on average it was four times higher than in the forest soils. It is interesting that the activity of all the investigated enzymes at the site 200 m away from the emitter was significantly higher than at the site located 400 m away from the emitter. The soil at the site located 200 m from the emitter had relatively high activity of dehydrogenase. The only soil in which that activity was higher was that of the cultivable field. The activity of dehydrogenase, phosphatase, and protease in the forest soil was significantly lower, by 20-30%, than in the soil of the cultivable field.

Table 4. Enzymatic activity of the soils

Distance From NP (m)	DhA	PhA	UA	PA
50	1.22	4.3	333.8	3.4
200	1.98	7.6	344.6	5.7
400	1.43	6.5	199.7	4.9
600	1.67	8.2	86.3	6.2
1000	1.72	9.2	72.8	7.9
2000 – forest soil	1.85	10.7	94.6	8.3
2000 – arable soil	2.42	12.8	347.2	11.7
$LSD_{0.05}$	0.09	0.3	2.8	0.2

DhA – Dehydrogenase activity in μg TPF g^{-1} 24 h^{-1}

PhA – Phosphatase activity in μg p-nitrophenol g^{-1} h^{-1}

UA – Urease activity in μg $N\text{-}NH_4^+$ g^{-1} 24 h^{-1}

PA – Protease activity in μg tyrosine g^{-1} h^{-1}

Table 5 contains values of correlation coefficients between the activity of soil enzymes and the content of heavy metals in the investigated soils. Stated was negative correlation between the activity of dehydrogenase and the content of Zn in the soil at the level of $p=0.05$. The activity of phosphatase and protease was negatively correlated with the content of Cd, Cu, Pb, Zn ($p=0.01$) and Ni ($p=0.05$) in the soil. Positive correlation occurred between the activity of urease and the content of Cd, Cu, Ni, Pb ($p=0.01$) and Zn ($p=0.05$) in the soils.

Table 6 contains data of correlation coefficient between the content of heavy metals in the investigated soils and their physical-chemical properties. The content of the investigated heavy metals positively correlated with the soil pH values and negatively correlated with exchange adsorption of the soils (CEC). The content of Cd, Cu, Ni, and Pb was positively correlated with the total of exchangeable bases (TEB) and with the base saturation (BS).

Table 5. Correlation coefficients between enzymatic activity and the heavy metal content in the soils (N=21)

Properties	DhA	PhA	UA	PA
Cd	n.s.	-0.78**	0.79**	-0.73**
Cu	n.s.	-0.79**	0.77**	-0.72**
Ni	n.s.	-0.56*	0.91**	-0.46*
Pb	n.s.	-0.83**	0.77**	-0.78**
Zn	-0.43*	-0.81**	0.66*	-0.73**

** significant at p = 0.01; * significant at p = 0.05; n.s. – no significant

Table 6. Correlation coefficients between the heavy metal content and physical-chemical properties of the soils (N=21)

Properties	pH_{H2O}	pH_{KCl}	TEB	CEC	BS
Cd	0.77*	0.76*	0.57*	-0.46*	0.65*
Cu	0.73*	0.73*	0.53*	-0.45*	0.61*
Ni	0.80*	0.78*	0.60*	-0.59*	0.72*
Pb	0.78*	0.78*	0.54*	-0.50*	0.63*
Zn	0.56*	0.56*	n.s.	-0.46*	n.s.

** significant at p = 0.01; * significant at p = 0.05; n.s. – no significant

DISCUSSION

The received results indicate the processes of chemical and biological degradation of the investigated soil proved by losses in the organic matter, deviation of the ion balance in the environment, disturbances in the intensity of the biochemical processes, and high acidification of the forest soils. Despite the decreasing for the last few years industrial emission the investigated ecosystem stays under constant pressure of the toxic factor [15]. In the investigated soils the effect of the heavy metal influence on the activity of the enzymes was related with the metal itself, the physical-chemical properties of the soil, and with the kind of the enzyme. This results probably from the great differentiation of sensitivity of the soil enzymes to environmental stress [14]. Heavy metals are considered inhibitors of the enzymatic activity in soils. Their inflow into the soil causes changes in quantity and quality of the soil microflora, which causes changes in the activity of the enzymes and leads to deviations in the proper metabolism of the soil components [7, 21, 29]. The activity of urease positively correlated with the content of the investigated heavy metals in the soils, which justifies the high activity of that enzyme in the sites that were highly polluted with heavy metals. The results of the research concerning the influence of the presence of heavy metals in the soil on the activity of urease are differentiated. The stimulating role of heavy metals on urease was stated by Dalton et al. [9] and Nowak et al. [24]. Opposite results were received by Badr El-Dim [2] and Frankenberger et al. [11]. The reason for such a differentiation in the research results could be the properties and the conditions of soils. Tolerance of the ecosystems to various changes in the content of the elements is different and depends mostly on the buffer properties of the environment. Łabuda and Niemira [23] emphasize that the capacity of the soil sorptive complex increases with an increasing content of the organic matter in the soil, which affects the activity of soil microorganisms and the mobility of heavy metals in soils. The activity of phosphatase and protease was the most sensitive indicator of the pollution of the investigated soils, which is proved by highly important values of the coefficients of negative correlation between the activity of those enzymes and the concentration of heavy metals in soils. In the case of dehydrogenase, the negative correlation of the activity of that enzyme was stated only in relation to the content of Zn in the soils. Research carried out by other authors [10, 12, 14] indicates that Zn is more toxic to soil microorganisms and enzymes than other heavy metals. The research carried out by the authors roved that in favorable conditions of the soil reaction (at the site 200 m away from the emitter, pH_{KCl} 6.2) the heavy metals accumulated in the soil stimulated its enzymatic activity. Leita et al. [21] observed an intensification of soil respiration caused by pollution with heavy metals. Those authors explain that phenomenon by higher energetic requirements of the living organisms in order for them to survive in the polluted soil. Mitigation of the consequences of harmful activity of heavy metals in the investigated soil could have occurred in result of the increased pH of the soil with greater content of heavy metals. The content of heavy metals positively correlated with the soil pH values, the total of exchangeable bases, and the base saturation. Research carried out by other authors [14, 25, 31] proved that heavy metal dust gets into the soil together with alkaline dust. The soil reaction is considered the main factor that decides on the assimilability of heavy metals in soils [1,13]. Exchangeable metal fractions, which are easily assimilable by soil microorganisms, dominate in soils with $pH<4.5$ and their quantity decreases with increasing pH of the soil [6]. High urease activity in the sites located closest to the Plant could have also been a result of higher content of urea (urease substrate). Investments made by the Nitrogen Plant "Puławy" S.A. in the years 1985-1999 on environment protection, limited the emission of pollution into the atmosphere.

Emission of fertilizer liquid (urea and ammonium nitrate) has been decreased by 85%, comparing to the emission in 1985, but it is still over 600 tons per year [19]. Dahm et al. [8] reported that fertilization of pine forest with urea stimulated the development of many groups of bacteria. An increase in the pH value was one of the reasons. The increase in the activity of dehydrogenase at the site located 200 m away from the emitter, where the content of Zn was high, could be explained by the fact that in favorable conditions of the soil reaction, Zn can stimulate the activity of dehydrogenase due to the presence of that element in its active center [5]. The phenomenon of the increasing activity of dehydrogenase in soils polluted with heavy metals was observed by Januszek [14].

High acidification of the forest soils was the reason for the decrease in their enzymatic activity. The significant impact of the pH on the enzymatic activity of the soils results from the specific reaction of microorganisms and plant roots on the concentration of hydrogen ions in the soil solution and from the unfavorable chemism of the soil solution in acid environment. As the acidity approaches the tolerance limit of the microorganism or higher plant species, their biological activity decreases, even to the 0 level. Enzymatic activity of the soil changes along with changing concentration of hydrogen ions due to reversible reactions of ionization or deionization of prototropic groups in the active center of the enzymatic protein and the irreversible denaturation of the enzyme. The solubility of the mineral components of the soil also increases, and their proportions in the solution change. In the forest soils the decrease in the pH value is caused by acid deposits, biological acidification, delusion of the basic components [26]. During the uptake of mineral components by tree roots, protons are mobilized, which increase the acidification of the soil solution. Moreover, when there is a long-lasting surplus of NH_4^+ ions in the soil environment and when the biological activity disappears, there are surpluses of H^+ protons which cause acid reactions [19]. Much lower acidification of the soil used for agricultural purposes resulting from the agrotechnical measures, caused an increase in its enzymatic activity.

The activity of the enzymes in particular study sites indicates that in the investigated ecosystem, in result of the accumulation, a new state of homeostasis was formed with new unbalanced energy circulation.

CONCLUSIONS

1. Changes in the intensity of the enzymatic activity in the soils of the studied sites depended on the ecological situation of the soil, as well as on individual properties of the enzymes.

2. The content of heavy metals (Cd, Cu, Ni, Pb, Zn) in the soil decreased significantly with the increasing distance from the Nitrogen Plant. Forest soils and the soil from the cultivable field showed natural content of the investigated heavy metals.
3. The activity of urease positively correlated with the content of the investigated heavy metals in the soils, which explains the very high activity of that enzyme in the sites highly polluted with heavy metals.
4. The activity of phosphatase and protease was the most sensitive indicator of pollution in the studied soils.
5. High acidification of the forest soils was the reason for the decrease in their enzymatic activity.
6. An important decrease in the content of organic carbon and total nitrogen was observed in the soils of the sites that lacked natural plant cover and were located closest to the Nitrogen Plant.
7. The received results indicated the processes of chemical and biological degradation of the studied soils proved by losses of the organic matter, disturbances in the intensity of the biochemical processes, and high acidification of the forest soils.

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AKTYWNOŚĆ ENZYMATYCZNA GLEBY JAKO WSKAŹNIK ZANIECZYSZCZENIA ŚRODOWISKA

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S t r e s z c z e n i e. Badania zlokalizowano w rejonie oddziaływania Zakładów Azotowych „Puławy" S.A. (51^0 25' N; 21^0 57' E). Punkty badawcze wytypowano w kierunku północno-wschodnim (na przeważającej róży wiatrów) w odległości 50, 200, 400, 600, 1000 i 2000 m od Zakładów. W punkcie odległym o 2000 m od ZA próbki gleby pobrano na siedlisku boru świeżego oraz na przyległym polu uprawnym spod pszenicy ozimej. Gleba o składzie granulometrycznym piasku słabo gliniastego (zawartość frakcji 1-0,1 mm 83-87%; frakcji 0,1-0,02 mm 9-12% i frakcji < 0,02 mm 3-5%) charakteryzowała się wadliwymi właściwościami sorpcyjnymi: małą pojemnością sorpcyjną, niską sumą kationów zasadowych i małym wysyceniem kompleksu sorpcyjnego kationami zasadowymi. W okresie prowadzonych badań obserwowano spadek wartości pH gleby wraz ze wzrostem odległości od ZA. Najmniejszą aktywnością dehydrogenaz, fosfataz i proteazy cechowała się gleba w punkcie położonym najbliżej ZA (50 m), co wiązało się z silnym zanieczyszczeniem środowiska glebowego metalami ciężkimi. Przeciwne tendencje odnotowano w przypadku ureazy. Aktywność tego enzymu była największa w punktach badawczych położonych najbliżej ZA. Na uwagę zasługuje fakt, że aktywność wszystkich badanych enzymów w glebie z punktu oddalonego o 200 m od ZA była istotnie większa w porównaniu z aktywnością tych enzymów w glebie z punktu oddalonego o 400 m od ZA. W obrębie punktu badawczego odległego o 2000 m od ZA aktywność enzymatyczna gleby leśnej była istotnie mniejsza niż gleby pola uprawnego.

S ł o w a k l u c z o w e : gleba , aktywność enzymatyczna, zanieczyszczenia.