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SEASONAL CHANGEABILITY OF ENZYMATIC ACTIVITY IN SOILS OF SELECTED FOREST SITES

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Abstract. Seasonal changeability of soil enzyme activity has not been entirely known. The aim of the paper was presenting the seasonal changeability in enzyme activity in fresh forest sites. From 10/06/2007 to 20/06/2008 samples were taken seven times at 6-weeks' intervals from two sample plots representing sites Lśw, LMśw, BMśw and one for Bśw site. Activity of dehydrogenase, protease and urease have been marked in the soil samples. As a result of conducted research the minimum value for enzymatic activity was found in October 2007 and April 2008. Maximum values for the activity of studied enzymes were noted in January and June 2007 and 2008, while no statistically significant differences were found between enzymatic activity marked in June 2007 and June 2008.

Key words: seasonal changeability, enzymatic activity, forest sites

INTRODUCTION

Seasonal changeability in enzyme activity is not entirely known. Skujinš [1967] reports that seasonal fluctuation of activity is generally low. Dormaar et al. [1984] pointed out that the activity of dehydrogenase, phosphatase and urease increases during winter and decreases in summer. Rastin et al. [1988] believes that the maximum activity occurs in spring. Research on seasonal changeability of enzymatic activity was also conducted by Januszek [1993], as well as Koper and Piotrowska [1996]. Januszek [1993] noted more or less evident maximum of activity in summertime in mountain soils in respect of all the studied enzymes. According to Koper and Piotrowska [1996] there are two periods of increased development of soil microorganisms and thus the increase in intensity of biochemical transformations in the year: the first one in spring – together with the arrival of higher temperatures, the latter in autumn after being supplied new organic matter. Sardans et al. [2008] noted a positive correlation between enzymatic activity and winter temperatures. In spring and autumn they did not note a connection between temperature, moisture and enzymatic activity. According to Kubista [1982] temperature and moisture are significant factors affecting the activity of dehydrogenases. Apart from

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temperature and moisture the activity and durability of enzymes in soil are controlled by pH, flora, organic matter and silt mineral resources [Trasar-Cepeda and Gil-Sotres 1987, Tarafdar and Jungk 1987, Kandeler and Eder 1993].

The aim of the paper was marking the enzymatic activity in dehydrogenases, proteases and urease in the soils of selected forest sites and presenting seasonal changeability of enzyme activity. The conducted research was also to explain what the changeability of enzymatic activity depends on in the course of the year and in what way enzymatic activity varies in trophically differentiated soils.

RESEARCH MATERIAL AND METHODS

The research was carried out at the Przedbórz forest district which is characterised by high diversity of lowland sites in the relatively small area. The forest district has not been changed through the activity of industrial pollutions; it belongs to zone 0, free from damages.

While choosing the location of sample plots the following site – forming factors were considered – geological substrate, terrain formation and soil. The plots were localized in older class stands. Two plots were established in Lśw, LMśw and BMśw sites, one in Bśw site. In all the sample plots a detailed description of soil profile was conducted, from every genetic level samples were taken to perform the basic markings of physical and chemical properties. In the samples granulometric composition was marked with Bauyoucosa-Casagrande aerometric method in Prószyński's modification, soil reaction was marked with potentiometric method in water and 1M KCl, hydrolytic acidity was marked with Kappen's method, exchangeable acidity and aluminium with Sokolov's method, total nitrogen content with Kjeldahl's method, organic carbon content with modified Tiurin's method, with calculation of C/N ratio, alkaline cations content in 1M ammonium acetate with calculation of the degree of soil saturation with cations that are alkaline in nature (V%).

From 10/06/2007 to 20/06/2008 at six-week intervals samples were taken seven times from two plots representing the sites Lśw, LMśw, BMśw and one from the Bśw site. 20 samples of natural moisture were taken at one time. Samples taken for marking the enzymatic activity constituted a collective sample consisting of soil from the pit and from 8 places surrounding the pit.

Dehydrogenase activity was marked with Lenhard's method according to the Casidy procedure, expressing their activity in Triphenyl formazan milligrams (TFF) for 100 g of soil within 24 hours. The method is known as "the TTC test", 3 per cent solution of triphenyltetrazolium chloride (TTC). For washing out the formazan gathered in soil ethyl alcohol contaminated with methanol was used. Protease activity was marked with Hoffman and Teicher method [Haziev 1976], with the application of 2-per cent solution of gelatin as substrate, expressing enzyme activity in $N\text{-}NH_2$ milligrams for 100 g of soil during 20 hours. Urease activity was marked with Tabatabai and Bremner's method (1972) [Alef and Nannipieri 1995], expressing enzyme activity in μg $N\text{-}NH_4$ for 1 g of soil during 2 hours.

Mean monthly values of air temperature, minimum air temperatures and mean ground temperatures at the depth of 5 cm as well as monthly totals of atmospheric precipitation from meteorological stations in Łódź and Kielce were used. The data were taken from Agrometeorological Bulletins.

Statistical data analysis was performed Using the program Statistica 8, differences between the means from two samples were tested with U Mann-Whitney test.

RESULTS

Arenosols soils prevailed at the plots selected for the study. In Lśw site district cambisols and haplic arenosols were diagnosed (sample plots No. 1 and 5), in LMśw site haplic arenosols (sample plot No. 3 and 7), in BMśw site proper haplic arenosols and gleyic podzols (sample plot No. 2 and 4) and in Bśw site haplic podzols was diagnosed (sample plot No. 6). In forest site stands pine prevailed, in BMśw site – pine with addition of oak and beech, and in Bśw site pine was the only species. Broadleaf species (oak, beech, hornbeam) and fir prevailed in the stands of LMśw and Lśw sites.

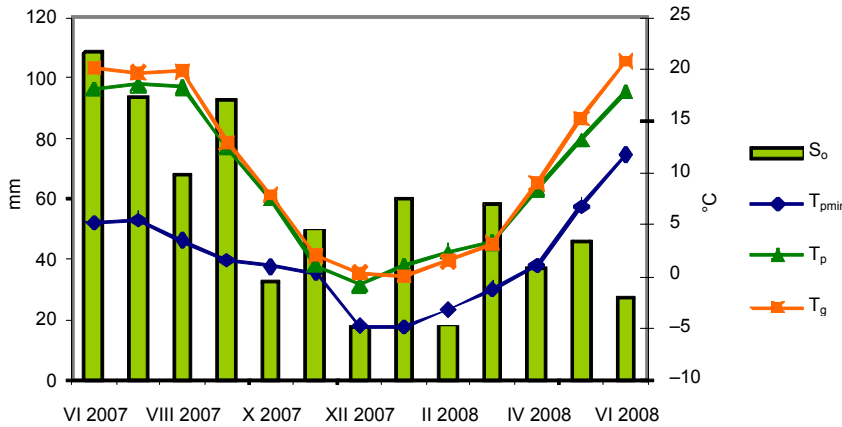

Fig. 1. Meteorological characteristics of sample plots: S_o – mean total precipitation, T_p – mean monthly air temperature, T_{pmin} – minimal air temperature, T_g – mean ground temperature at the depth of 5 cm. Mean value data from meteorological stations in Łódź and Kielce

Rys. 1. Charakterystyka meteorologiczna powierzchni badawczych: S_o – średnia suma opadów atmosferycznych, T_p – średnia miesięczna temperatura powietrza, T_{pmin} – minimalna temperatura powietrza, T_g – średnia temperatura gruntu na głębokości 5 cm. Dane uśrednione ze stacji meteorologicznych w Łodzi i w Kielcach

In case of dehydrogenase activity (Fig. 2) the first minimum of activity was noted in October 2007, another one in April 2008. The highest activity was marked in January 2008, slightly lower in August 2007 and June 2008. High fluctuation was noted in fertile broadleaf forest sites (Lśw, LMśw), activity in those sample plots takes the values from 12.2 mg TFF·100 g of soil^{-1}·24 h^{-1} in April 2008 to 37.7 mg TFF·100 g of soil^{-1}·24 h^{-1} in January 2008 (amplitude 25.5). BMśw and Bśw sites were characterised by lesser changeability and the dehydrogenase activity minimum amounted to 0.96 mg TFF·100 g of soil^{-1}·24 h^{-1} in April 2008, and maximum – 16.35 mg TFF·100 g of soil^{-1}·24 h^{-1} in January 2008 (amplitude 15.39).

Table 1. Chemical soil properties
Tabela 1. Właściwości chemiczne gleb

Profile number Numer profilu	Horizon, cm Poziom, cm	Textural group Grupa mechaniczna	pH in H_2O pH w H_2O	pH in KCl pH w KCl	S	T	V	C_{org}	N_{og}	C/N
					$cmol_{(+)}\ kg^{-1}$			%		
1	2	3	4	5	6	7	8	9	10	11	12
5	A	2-7	pgśr	4.6	3.5	1.2	13.2	9	2.5	0.2	16.9
	ABbr	7-23	pgśr	4.7	3.8	0.4	5.1	8	0.8	0.1	13.9
	Bbr	23-46	pgśr	4.7	4.0	0.4	3.7	11	0.5	0.0	13.1
	BbrCgg	46-55	pgśr	4.7	3.9	0.4	2.9	15			
	IICgg	55-170	gpi	5.5	4.0	8.2	10.4	78			
6	Ofh	2-8	n.o.	4.0	3.0	3.8	37.0	10	10.6	0.5	19.7
	A	8-16	plgr	4.3	3.6	1.4	9.4	15	2.0	0.1	17.9
	ABv	16-23	psśr	4.7	4.0	0.2	2.2	11	1.3	0.1	15.9
	Bv	23-53	plśr	4.8	4.2	0.2	2.5	9	0.4	0.0	14.8
	BvC	53-80	psśr	5.1	4.3	0.4	1.7	22			
	IIC	80-200	pgśr	4.6	3.8	0.9	3.6	24			
7	Ofh	1-3	pgśr	4.3	3.4	1.0	10.7	10	2.8	0.2	17.4
	ABbr	3-9	pgśr	4.6	3.8	0.5	5.7	8	1.4	0.1	17.5
	Bbr_1	9-35	pgśr	4.8	4.0	0.3	3.3	9	0.8	0.0	19.8
	Bbr_2	35-50	pgśr	4.9	4.2	0.2	2.7	8			
	BC	50-97	pgdr	5.4	4.2	0.9	2.1	43			
	IICgg	97-145	gp	5.5	3.7	4.9	6.8	73			
	IIICgg	145-200	pgdr	5.7	4.2	2.0	3.2	63			
8	Ofh	3-10	n.o.	3.9	2.9	5.9	58.8	10	21.5	0.8	27.2
	AEes	10-20	plgr	3.8	3.0	0.3	4.6	7	0.8	0.1	16.1
	Eesg	20-34	plgr	4.0	3.2	0.2	3.5	6	0.6	0.0	21.3
	Bhfe	34-55	plśr	4.7	4.1	0.4	4.8	9	0.9	0.1	14.5
	BfeGo	55-80	plgr	4.7	4.2	0.2	2.1	8			
	CGr	80-200	plśr	4.9	4.4	0.2	1.2	15			
9	A	3-9	gp	3.7	3.0	0.5	13.1	4	2.1	0.2	14.1
	ABvBbr	9-26	gp	4.4	3.9	0.3	6.2	5	1.3	0.1	14.9
	$BvBbr_1$	26-54	gp	4.2	4.8	0.4	5.1	8	0.7	0.0	16.5
	$BvBbr_2$	54-67	pgśr	4.3	4.0	0.3	3.7	9	0.7	0.0	16.7
	IIBvCgg	67-77	pgśr	4.2	3.6	0.4	5.7	7	0.2	0.0	10.7
	IICgg	77-180	pg	4.2	3.8	0.3	4.5	8			
14	Ofh	2-5	n.o.	3.6	2.7	3.2	40.8	8	11.4	0.4	30.7
	AEes	5-19	plśr	3.6	2.9	0.3	3.8	8	0.9	0.0	23.5
	Bfe	19-47	plśr	4.4	4.0	0.2	3.9	4	0.8	0.8	1.0
	BfeC	47-95	plśr	4.6	4.2	0.2	2.0	8			
	C	95-200	plśr	4.5	4.3	0.2	1.6	11			

Table 1 – cont. / Tabela 1 – cd.

1	2	3	4	5	6	7	8	9	10	11	12
15	Ofh	1-3	pggr	4.0	3.2	2.3	23.0	10	6.3	0.4	16.9
	ABbr	3-21	pgśr	3.7	3.1	0.4	10.2	4	1.9	0.1	19.1
	BvBbr	21-38	pgśr	4.4	3.8	0.4	3.8	11	0.3	0.0	11.7
	C_1	38-85	psgr	4.5	3.8	0.3	2.8	12			
	IIC_2gg	85-180	plśr	4.8	4.0	0.3	1.4	18			

Textural group: pl – loose sandy soil, pg – medium sand, ps – coarse sandy soil, gp – sandy loam.
S – sum of exchangeable bases, T – hydrolitic sorption capacity, V – degree of base saturation, C_{org} – organic C, N_{og} – total N.

Grupa mechaniczna: pl – piasek luźny, pg – piasek gliniasty, ps – piasek słabogliniasty, gp – glina piaszczysta.
S – suma zasadowych kationów wymiennych, T – całkowita pojemność sorpcyjna, V – stopień wysycenia kompleksu sorpcyjnego kationami zasadowymi, C_{org} – węgiel organiczny, N_{og} – azot ogólny.

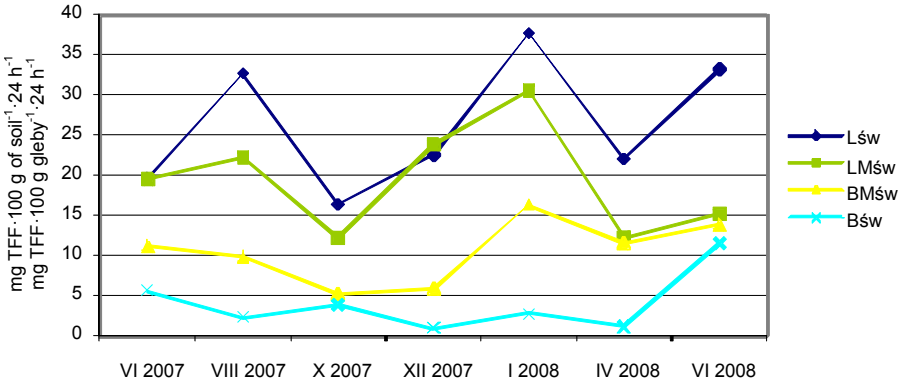

Fig. 2. Mean dehydrogenase activity (mg TFF·100 g of soil^{-1}·24 h^{-1}) at humus levels of soils in studied sites (Lśw, LMśw, BMśw, Bśw)
Rys. 2. Średnia aktywność dehydrogenaz (mg TFF·100 g gleby^{-1}·24 h^{-1}) w poziomach próchnicznych gleb badanych siedlisk (Lśw, LMśw, BMśw, Bśw)

The lowest protease activity (Fig. 3) like in case of dehydrogenase activity, was marked in October 2007 and in April 2008. The highest protease activity was marked in January 2008 and in June 2007 and 2008. High variability in protease activity at humus level was found in the soils of BMśw and Bśw sites (min. protease activity 68.0 mg N-NH_2·100 g of soil^{-1}·20 h^{-1} in October 2007, max. 312.9 mg N-NH_2·100 g of soil^{-1}·20 h^{-1} in January 2008). Lower protease activity changeability at humus level was noted in Lśw and LMśw sites soils (min. protease activity 62.1 mg N-NH_2·100 g of soil^{-1}·20 h^{-1} in April 2008, max. 241.3 mg N-NH_2·100 g of soil^{-1}·20 h^{-1} in June 2007).

The lowest urease activity was marked in October 2007 and in April 2008 in all the selected sites (Fig. 4). The highest urease activity was however marked in January 2008 in sites Lśw and LMśw, as well as in August and June 2008 in Lśw site. High fluctuation of enzymatic activity was found in humus level of broadleaf forest site soils (min. urease activity 5.49 µg NH_4-N·1 g of soil^{-1}·2 h^{-1} in October 2007, max. 18.29 µg NH_4-

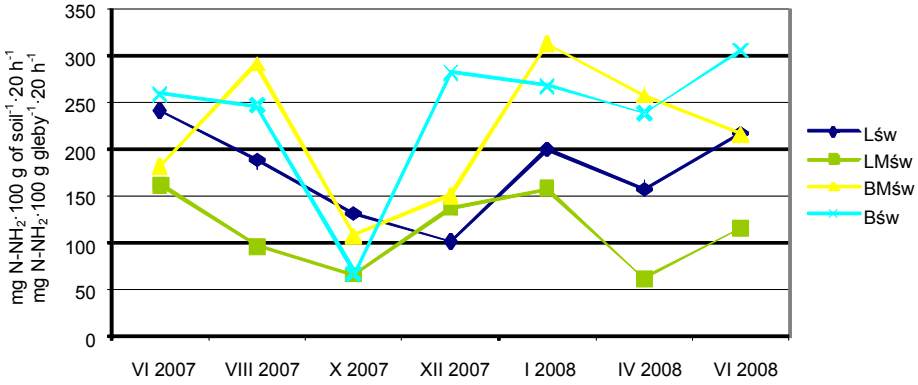

Fig. 3. Mean protease activity (mg N-NH_2·100 g of soil^{-1}·20 h^{-1}) at humus levels of soils in studied sites (Lśw, LMśw, BMśw, Bśw)

Rys. 3. Średnia aktywność proteaz (mg N-NH_2·100 g gleby^{-1}·20 h^{-1}) w poziomach próchnicznych gleb badanych siedlisk (Lśw, LMśw, BMśw, Bśw)

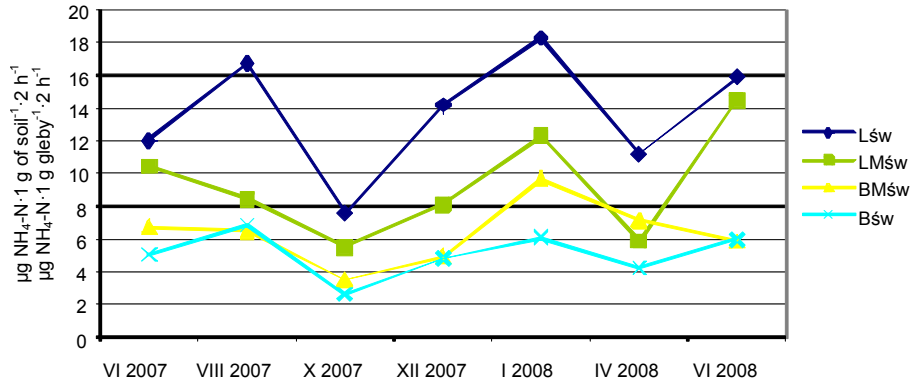

Fig. 4. Mean urease activity (µg NH_4-N·1 g of soil^{-1}·2 h^{-1}) at humus levels of soils in studied sites (Lśw, LMśw, BMśw, Bśw)

Rys. 4. Średnia aktywność ureazy (µg NH_4-N·1 g gleby^{-1}·2 h^{-1}) w poziomach próchnicznych gleb badanych siedlisk (Lśw, LMśw, BMśw, Bśw)

-N·1 g of soil^{-1}·2 h^{-1} in January 2008), lower changeability occurred in the soils of Bśw and BMśw sites (min. urease activity 2.67 µg NH_4-N·1 g of soil^{-1}·2 h^{-1} in October 2007, max. 9.69 µg NH_4-N·1 g of soil^{-1}·2 h^{-1} in January 2008).

In the course of the research the highest enzyme activity was noted in humus levels, the activity dropped together with the depth. The general trend in the activity of particular enzymes in soil profiles covered by the research from June 2007 to June 2008 was similar to the one rendered in Figure 5. In deeper genetic levels the differences in enzyme activity in particular months were lower than in humus levels. Minimum activity of dehydrogenases, proteases and urease in deeper soil levels was noted in October 2007 and in April 2008. A clear increase in the activity was observed in June 2007 and 2008 and in January 2008.

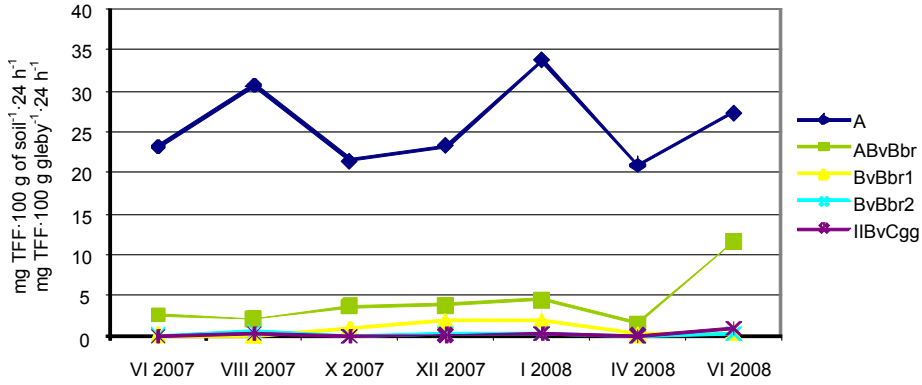

Fig. 5. Dehydrogenase activity (mg TFF·100 g of soil^{-1}·24 h^{-1}) at soil genetic levels at sample plot No. 5 (Lśw)

Rys. 5. Aktywność dehydrogenaz (mg TFF·100 g gleby^{-1}·24 h^{-1}) w poziomach genetycznych gleby na powierzchni nr 5 (Lśw)

Table 2. Comparison of enzymatic activity of dehydrogenases and urease within forest site types (data as of June 2007 and 2008)

Tabela 2. Porównanie aktywności enzymatycznej dehydrogenaz i ureazy w obrębie typów siedliskowych lasu (dane z czerwca 2007 i 2008 roku)

Enzymatic activity Aktywność enzymatyczna	Statistics value U Wartość statystyki U	Value p Wartość p
Dehydrogenase activity in Bśw site soils Aktywność dehydrogenaz gleb siedliska Bśw	53.0	0.6457
Urease activity in Bśw site soils Aktywność ureazy gleb siedliska Bśw	44.0	0.2934
Dehydrogenase activity in BMśw site soils Aktywność dehydrogenaz gleb siedliska BMśw	22.0	0.7982
Urease activity in BMśw site soils Aktywność ureazy gleb siedliska BMśw	24.5	0.9490
Dehydrogenase activity in LMśw site soils Aktywność dehydrogenaz gleb siedliska LMśw	32.0	0.4799
Urease activity in LMśw site soils Aktywność ureazy gleb siedliska LMśw	29.0	0.3313
Dehydrogenase activity in Lśw site soils Aktywność dehydrogenaz gleb siedliska Lśw	40.0	1.000
Urease activity in Lśw site soils Aktywność ureazy gleb siedliska Lśw	30.0	0.3772

Using U Mann-Whitney test no statistically significant differences were found in the activity of dehydrogenases and urease within the studied forest site types in June 2007 and 2008 despite the differences in the mean total atmospheric precipitation and minimal air temperature (Fig. 1). Testing was carried out at a bigger group of soil samples (36 items) taken in June 2007 and 2008. The samples represented sites Bśw, BMśw, LMśw and Lśw.

DISCUSSION

Seasonal variability of enzymatic activity was noted in the paper. Baligar et al. [1991], Koper and Piotrowska [1999], Trasar-Cepeda et al. [1998] and Wick et al. [2002] made similar observations. Seasonal changeability of enzyme activity depends on numerous factors, such as aeration, moisture and temperature of soil, flora and soil microflora [Kiss et al. 1975, Rastin et al. 1988]. Januszek [1993] studied seasonal changeability in enzymatic activity in basic types of forest humus in Western Beskidy Mountains. High values of enzymatic activity were found in all the four seasons, which could be the result of such phenomena as: temperature, moisture, vegetation, microflora, inflow of fresh organic matter to the soil and intense washing out of enzymes, cryoactivation of enzymes, destructive action of the phenomenon of soil colloids and microorganisms freezing and defreezing. Fenner et al. [2005] noted an increase in enzymatic activity from winter to autumn which is related to the growth of plants and microbiological activity during the vegetation season and then with the period of growing old and atrophy. Eivazi and Tabatabai [1990] stated that activity of β-glucosydase differs depending on soil moisture. According to Kubista [1982], Brzezińska et al. [2001], temperature and moisture are significant factors affecting dehydrogenase activity.

Meteorological conditions (moisture, temperature) have considerable influence on forming enzymatic activity which is proved by the obtained results. In case of three enzymes of different characteristics minima and maxima were noted at the same time. Minimum value of enzymatic activity was found for dehydrogenases, urease and proteases in October 2007 and April 2008 . Maximum values of activity in the studied enzymes were noted in June 2007 and 2008 and in January 2008. No statistically significant differences were found between enzymatic activity marked in June 2007 and June 2008. Garbolińska [2008] noted maximum activities of urease and phosphatase in the summer although they slightly differed at particular sites. It was probably determined by differences in soil water-air conditions at those sites making optimal conditions for biochemical processes at particular levels of the studied soils be formed in different type of weather. The highest seasonal fluctuations occurred in the activity of acid phosphatase, while the most even activity was revealed by cellulase and urease. Maximum enzymatic activity in winter was noted by Dormaar et al. [1984] who pointed out that the activity of dehydrogenases, phosphatases and urease clearly increases in winter and decreases in summer. Sardans et al. [2008] did not note a relation between temperature, moisture and enzymatic activity in spring and autumn. According to Myśków [1981] the time of taking samples for enzymatic markings should be in the period when soil is in the state of equilibrium and favourable climatic conditions appear.

The process of soil freezing and defreezing could be important in forming the obtained results. The increase in enzymatic activity noticed in January was caused by higher number of microorganisms. As a result of soil freezing microorganisms die, thus increasing the food basis for spore forms. After the temperatures below zero disappear, there occurs an increase in the number of microorganisms. The results obtained by Januszek [1999] in research into the influence of soil freezing and defreezing on its enzymatic activity point to a complex enzyme and microorganism sorption and desorption mechanism in soil, as well as to the complex course of enzymatic reactions. The author observed a differentiated influence of freezing and defreezing of the studied soils on enzyme activity, depending both on individual features of the studied enzyme and on the properties of the studied soils. Winter et al. [1994] estimated that as a consequence

of soil freezing and defreezing the number of microorganisms decreases by 30-50%, but there are also reports on the increase in the number of bacteria and actinomycetes; such results were probably obtained due to the application of improved extraction causing dispersion of soil aggregates.

High fluctuation in the activity of dehydrogenases and urease during the year was noted in fertile broadleaf forest sites (Lśw, LMśw), and lower changeability appeared in coniferous sites BMśw and Bśw. In coniferous forest sites there forms a system of overburden humus of considerable depth – mor humus characterised by low pH and limited soil organism activity, as a result of which the decomposition of organic remnants occurs slowly. Levels of overburden humus are less sensitive to unfavourable influence of natural factors. In broadleaf forest habitats mull humus soils prevailed, where microbiological and biochemical activity are higher and where organic substance decomposition occurs quickly.

High enzyme activity marked in own sample plots in the summer months may be related to the increased development of soil microorganisms and thus with the increase in the intensity of biochemical transformations. In the summer there is a period of a balanced conditions in soil which above all is connected with temperature, moisture and air conditions in the soil which significantly affect microorganisms. Microorganisms creating specific enzymes participate in all the stages of particular phases of transformations in nitrogen compounds [Wyczółkowski et al. 2006]. Weather conditions influence the course of organic substance mineralization processes and its immobilization. According to Czępińska-Kamińska et al. [1999] the content of particular nitrogen forms is associated with the microorganism activity and differs during the vegetation season.

SUMMARY

1. The conducted research revealed the differentiation of enzymatic activity during the year. The minimum of enzymatic activity was noted in October 2007 and in April 2008, while maximum values in June 2007 and 2008 as well in January 2008.

2. Within the forest site and soil types enzymatic activity showed diversity increasing with the increase in soil and site fertility.

3. Enzymatic activity depends on temperature, soil moisture and flora. The process of freezing and defreezing significantly influences soil enzyme activity through affecting soil microorganisms and their activity.

4. The lowest fluctuations of enzymatic activity appear in the summer months when soil is in the state of equilibrium (temperature, moisture), which is conducive to the development of soil microorganisms and leads to the increase in biochemical transformation intensity.

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SEZONOWA ZMIENNOŚĆ AKTYWNOŚCI ENZYMATYCZNEJ GLEB WYBRANYCH SIEDLISK LEŚNYCH

Streszczenie. Sezonowa zmienność aktywności enzymów glebowych nie jest poznana do końca. Celem pracy było przedstawienie sezonowej zmienności aktywności enzymatycznej na świeżych siedliskach leśnych. Od 10.06.2007 roku do 20.06.2008 roku zostały pobrane próbki siedmiokrotnie, w odstępach 6-tygodniowych, z dwóch powierzchni reprezentujących siedliska Lśw, LMśw, BMśw i jednej – siedliska Bśw. W próbkach gleby oznaczono aktywność dehydrogenaz, proteaz i ureazy. W wyniku przeprowadzonych badań stwierdzono minimum wartości aktywności enzymatycznej w październiku 2007 i kwietniu 2008 roku. Maksymalne wartości aktywności badanych enzymów zanotowano w styczniu i czerwcu 2007 i 2008 roku, nie stwierdzono statystycznie istotnych różnic pomiędzy aktywnością enzymatyczną oznaczoną w czerwcu 2007 roku i w czerwcu 2008 roku.

Słowa kluczowe: sezonowa zmienność, aktywność enzymatyczna, siedliska leśne

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