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The effect of the use of a soil improver based on waste brown coal on the enzymatic activity of soil in the cultivation of Paulownia hybrids (*Paulownia* Siebold & Zuccarini, 1835)

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Abstract: *The effect of the use of a soil improver based on waste brown coal on the enzymatic activity of soil in the cultivation of Paulownia hybrids (Paulownia Siebold & Zuccarini, 1835).* An important element in controlling the condition of the soil and the plants grown on it are tests of the enzymatic activity of the soil matrix. One of the greatest advantages of using enzyme tests is the ability to make an assessment that also includes other non-measurable factors that affect soil health and condition. The diagnosed changes in soil enzymatic activity are the best parameter for determining the biochemical processes taking place there. This article describes the enzymatic activity of lessive soils on which the Paulownia hybrid variety is cultivated and a soil improver based on waste brown coal is used

INTRODUCTION

For decades, intensive agricultural production has intensified soil degradation processes. Any imbalances in the soil ecosystem are most often regulated by the use of various measures to improve the quality of the soil, and in the case of undesirable plants also pesticides. During the last half-century, therefore, special attention was paid to the assessment of soil not only through its ability to produce green mass, but also the safety of plants grown there, as well as human and animal health [Skuijns, 1967; Šarapatka et al., 2002; Torstensson et al., 1998]. At present, tests of the enzymatic activity of the soil matrix are an important element in controlling the condition of the soil and the plants grown there [Kucharski, 1997]. One of the greatest advantages of using enzyme tests is the possibility of making an assessment that also includes other non-measurable factors affecting the condition and condition of the soil [Kieliszewska-Rokicka 2001]. The diagnosed changes in soil enzymatic activity are the best parameter for determining the biochemical processes taking place there [Gostkowska et al. 1998]. In order to learn the proper elements of biochemical changes, the most frequently used enzymes are those that can inform about stressors, and thus inform about their intensity [Gupta and Germida 1988; Clarholm, 1993] and changes in their use. [Adams 1992].

The soil is one of the systems in which all biochemical changes take place through enzymatic processes taking place in it. In soil, there are free enzymes called exoenzymes, which are most often secreted from living cells and work outside the cells, and endoenzymes, which to a large extent only work inside the cells [Stręk and Telesiński, 2015]. Most of those added to the soil are broken down by proteinases, and the intermediates produced as a result of decomposition are incorporated into the organic substance [Skuijns, 1976]. Hence, the soil matrix can be considered as a system of biohumus and mineral parts that carry a load of non-released enzymes [McLaren, 1975]. In the assessment of the enzymatic activity of soils, the division proposed by Roberge in 1978, as well as the division of the Bioechemical Union, should be considered:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

Dehydrogenases belonging to the group of transferases work by detaching electrons and protons from organic compounds [Włodarczyk, 2000; Brzezińska et al., 1998; Gliński et al. 1986]. Like catalases, dehydrogenases are interdependent with other soil-shaping factors [Nannipieri, 2002]. Several of them carry functional groups, which in turn have a great influence on the synthesis of oligosaccharides [Hofmann, 1963]. From the point of view of improving the quality of soils, urease is of the greatest importance, which is of great importance in fertilization and the transformation of urea in the soil. As a result of hydrolysis, urea introduced into the soil is transformed into ammonium carbonate. As a result of further degradation to ammonia in the form of gas and carbonic acid, this nitrogen can be taken up by plants [Kucharski, 1997]. Due to the fact that most of the fertilising and soil improving substances currently used in agriculture are based on urea, the importance of urease as a diagnostic enzyme has increased significantly. However, its excessive activity can lead to too rapid decomposition of urea, which usually leads to huge nitrogen losses in the soil.

Phosphatases, in turn, include a whole group of enzymes responsible for the hydrolysis of the anhydride form of orthophosphate acid and esters [Eivazi and Tabatabai, 1977]. The group of these enzymes is extremely important when considering phosphorus fertilization. As a result of decomposition, phosphorus breaks down into inorganic phosphates, which can be directly taken up by plants [Speir and Ross, 1978]. Due to the number of ester bonds, they have been divided into 3 groups: phospho-monoesterase, phosphodiesterase and phosphotriesterase.

Phosphomonoesterases are of the greatest importance for agriculture. The description of acid phosphatase is much more common in the literature due to the nature of the soil in the environment. Studies show that heavy metals and some trace elements in increased concentrations have an inhibitory effect on the activity of phosphatases. The activity of these enzymes, as well as others, is most often concentrated in the topsoil. Other enzymes belonging to this group also play a significant role in the phosphorus cycle: glycerophosphatase and phytase. This is due to the fact that most of the phosphorus in the environment is deposited in organic compounds, especially in phytin.

MATERIAL AND METHODS

The field experiment was carried out in 2018-2019 at the Agricultural Farm in Malechówko, Sławno poviat, Zachodniopomorskie voivodship.

The experiment was set up in a randomized sub-block design, in three replications, on plots with an area of 45 m^2.

Three factors were taken into account in the research:

Factor I - 3 fertilizing objects: control object (0) - without fertilization, Pg1 + N, Pg5 + N.

Factor II - three planting dates: early, medium late and late.

Factor III - two years of research: 2018 and 2019.

The test plant was a hybrid of Paulownia sp. Tomentosa x Fortuneii x Kawamii, (Z-07NT). A soil improver based on waste lignite from PAK Adamów, produced by INCO sp.z o.o. it was used in doses of 1 and 5 t.ha-1 (here in after referred to as Pg1 and Pg5) it contained 100 kg of N, 35 kg of P and 125 kg of K. For the top fertilization of Nom1 and Nom5, 60 kg of N was used in the form of urea (46% N).]

Planting of the test plant was performed on May 15, May 25, and June 5. Necessary care treatments were applied on the experimental plots.

Before setting up the experiment, a soil excavation was made to a depth of 150 cm. The profile is morphologically described.

• granulometric composition using the areometric method of Bouyoucos Casagrande as modified by Prószyński, in accordance with the Polish Standard PN-R-04033 [Polish Committee for Standardization 1998] and the Classification of Soils and Mineral Formation according to PTG 2008 [Polish Soil Science Society 2009],

• pH in a KCl solution with a concentration of 1 mol.dm-3 using the potentiometric method, using a combined electrode SG68-FK5-Kit and a SevenGo Duo pH meter (Mettler-Toledo) at a weight / volume ratio of soil to a solution of 1: 2.5,

• total carbon (Ct) and nitrogen (Nt) content on the Perkin Elmer CHNS / O Series II 2400 autoanalyzer with a thermal conductivity detector (TCD) and acetanilide as reference material,

• acid and alkaline phosphatase activity by the method of Tabatabai and Bremner [1969],

• urease activity by the colorimetric method according to Hoffmann and Teicher [1961],

• dehydrogenase activity by the colorimetric method according to Casida et al. [1964].

The experiment was carried out on medium soils with sandy loam grain composition. It was a soil belonging to the order of loamy soils (*P*), less than lesser soil (*PW*), subtype - sandy loam soil (*PWsp*) according to [Polish Soil Science Society 2011]. According to the IUSS Working Group WRB [2007, 2014], the soils on which the field experiment was conducted were classified as *Gleyic Luvisol* and *Albic Luvisol* (*Arenic*).

RESULTS

The activation and dynamics of the dehydrogenase reaction depend mainly on the concentration of enzymes present and participating in the soil reaction, substrate concentration, temperature in the soil profile, its pH value and the presence of inhibitors or activators of enzymatic processes [Russel, 2005].

The high activity of most soil enzymes promotes soil fertility, and thus affects the productivity and wide agricultural use of soils. According to Koper and Lemanowicz [2008], the presence and dynamics of enzymes in the soil profile can be an excellent source of information about biochemical changes that take place in the soil.

Analyzing the activity of dehydrogenases in sandy loam soil under the conditions of the conducted research (Table 1), significant differences in the activity of the tested enzymes in the soil were found under the influence of different fertilization and planting dates.

The applied fertilization significantly increased the activity of dehydrogenases reaching the highest mean values for the fertilization objects Pg1 + N and Pg5 + N, respectively 33.70 and 31.26 cm3 H2 · kgh-1. The soil in which the test plant was planted late and late was characterized by the highest activity of dehydrogenases, where Pg was applied at a dose of 1t ha-1 and topped with mineral nitrogen at a dose of 60 kg ha-1. objects without fertilizer applied. The highest activity of dehydrogenases was determined in soil collected from objects on which a medium-late planted plant was cultivated - 24.10 cm3 H2 · kgh-1, while the best effectiveness of dehydrogenases in soil equal to 34.23 cm3 H2 · kgh-1 was recorded during cultivation where the earliest planting date was with the addition of P1 + N. Statistical calculations showed a significant effect of the interaction of the applied fertilization and the planting date on the dynamics of dehydrogenase activity in the soil.

Table 1. Dehydrogenase activity (cm^3 H2 · kgh^{-1}) in sandy loaf soil (autumn 2019)

Fertilization objects /A/	Planting dates /B/			Averages for fertilization
	Early	Medium late	Late	
0	7,97	10,72	8,29	8,81
Nom1+N	34,23	31,78	34,10	33,70
Nom5+N	28,69	31,80	34,08	31,26
Averages for dates	23,63	24,77	25,49	24,59

$SSD_{0,05}$ for: using Pg / A / - 6,10; terms / B / - 1,38; B / A – 3,08; A / B –6,75
0 - control object (without improver). Pg1 + N - soil improver 1 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1. Pg5 + N - soil improver 5 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1.

In the studies of urease activity in the fallow soil (Table 2), significant differences in its activity were found under the influence of the examined factors and their interaction. Also with regard to the application of the soil improver, there were significant differences in the enzymatic decomposition of urea in the soil, reaching the highest level of process dynamics for the average of individual fertilizers, amounting to 45.18 and 48.16 mg N-NH4 + .kg.h-1 respectively for the Pg1 + N and Pg5 + N fertilization plants. As the research results show, the process of decomposition of ammonia in soil, which is the participation of the urease enzyme, is also significantly dependent on the interactions between individual planting dates and fertilizer objects.

The highest dynamics of urease activity was recorded for the early term (41.13 mg N-NH4 + .kg.h-1) and the lowest, amounting to 39.06 mg N-NH4 + .kg.h-1 for the medium late term. Heavy metals, including nickel, may play the role of catalysts or inhibitors in urease activity. It also occurs in ionic form in the active center of urease, therefore, depending on its content, it can act as a catalyst or act as an obstacle to its activation. As Wyszkowska and Wyszkowski [2004] claim, excess nickel has a negative impact on biological activity in soil also regarding urease.

Table 2. Urease activity (mg N-NH4 + $kg.h^{-1}$) in sandy loam soil (autumn 2019)

Fertilization objects /A/	Planting dates /B/			Averages for fertilization
	Wczesny	Średnio późny	Późny	
0	28,24	29,99	28,26	28,83
Nom1+N	47,52	44,29	43,42	45,18
Nom5+N	50,94	46,22	47,32	48,16
Averages for dates	42,23	40,17	39,67	39,83

$SSD_{0,05}$ for: using Pg / A / - 2.31; terms / B / - 0.85; B / A - 1.90; A / B –2.93
0 - control object (without improver). Pg1 + N - soil improver 1 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1. Pg5 + N - soil improver 5 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1.

Taking into account the results of the research carried out in the field of acid phosphatase activity in lessive soil for the adopted experimental conditions (Table 3), a significant influence of the applied doses of soil improver and the interaction of the examined factors were found. The essentially highest activity of acid phosphatase was determined in the soil collected from the control object (3.26 mmol pNP.kg.h-1). The lowest level of acid phosphatase was achieved using Pg1 (2.68 mmol pNP.kg.h 1). The increase in the organic matter in the soil, resulting from the applied organic fertilizers, significantly decreased the activity of acid phosphatase, which may indicate the presence of inhibitors in the organic matter that inhibit or limit the dynamics of enzymatic activity.

Table 3. Acid phosphatase activity (mmol pNP.kg.h^{-1}) in sandy loam soil (autumn 2019)

Fertilization objects /A/	Planting dates /B/			Averages for fertilization
	Early	Medium late	Late	
0	3,67	3,15	2,95	3,26
Nom1+N	2,55	2,63	2,86	2,68
Nom5+N	2,34	2,68	3,12	2,71
Averages for dates	2,80	2,74	2,82	2,79

$SSD_{0,05}$ for: using Pg / A / - 0,37; terms / B / - n.i.; B / A – 0,72; A / B – 0,79
0 - control object (without improver). Pg1 + N - soil improver 1 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1. Pg5 + N - soil improver 5 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1.

The applied fertilizers and the interaction of fertilization and cultivars significantly influenced the level of alkaline phosphatase activity (Table 4), similarly to the studies by Lemanowicz et al. [2013]. The highest level of alkaline phosphatase activity (0.53 mmol pNP · kg · h-1) was determined in soil using the soil improver Pg1 + N. It should be added, that the alkaline phosphatase activity in the soil, where the test plant was planted early, with the addition of Pg1 + N, was the highest (0.53mmol pNP · kg · h-1). The soil fertilized with Pg1 had the lowest activity of the analyzed enzyme (0.34 mmol pNP · kg · h-1)

Table 4. Alkaline phosphatase activity (mmol pNP.kg.h^{-1}) in sandy loam soil (autumn 2019)

Fertilizationn objects /A/	Planting terms /B/			Averages for ferttilization
	Early	Medium late	Late	
0	0,48	0,52	0,54	0,52
Pg1+N	0,50	0,58	0,52	0,53
Pg5+N	0,30	0,37	0,36	0,34
Averages for terms	0,45	0,42	0,42	0,43

$SSD_{0,05}$ for: using Pg / A / - 0,23; terms / B / - n.i.; B / A – 0,15; A / B –0,27
0 - control object (without improver). Pg1 + N - soil improver 1 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1. Pg5 + N - soil improver 5 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1.

The statistical analysis showed a significant diversification of the carbon content in the sandy loam soil under the influence of fertilization, cultivars and interaction of the examined factors. The test results presented in Table 5 fully confirmed the significant increase in the percentage of organic carbon in relation to the control object in the soil with the use of Pg1 + N and Pg5 + N.

The highest organic carbon content (1.42%) was determined in the soil where the early and medium late planted plants were grown using Pg5 + N and the highest carbon content (1.22%) in the soil using Pg1 + N medium early planted. On the basis of the research results presented in Table 5, significant differences were also shown in the content of organic carbon in the soil where individual maize varieties were grown. Significant differences were noted between early and mid-late planting.

Table 5. Organic carbon content (%) in sandy loam soil (autumn 2019)

Fertilization objects/A/	Planting terms /B/			Averages for fertilization
	Early	Medium late	Late	
0	0,72	0,72	0,74	0,73
Pg1+N	1,20	1,22	1,22	1,22
Pg5+N	1,42	1,21	1,42	1,35
Averages for terms	0,98	0,97	1,00	0,98

$SSD_{0,05}$ for: using Pg / A / - 0,03; terms / B / - 0,02; B / A – 0,04; A / B –0,05
0 - control object (without improver). Pg1 + N - soil improver 1 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1. Pg5 + N - soil improver 5 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1.

According to Gostkowska et al. [1998], the activity of various biochemical and microbiological processes that take place in soil has a fundamental impact on its fertility, and thus better agricultural use. As shown by the research results presented in Table 46, the applied fertilizers, cultivars and the interaction of the examined factors had a significant impact on the level of soil fertility. Mineral and organic fertilizers, the use of which, apart from the basic function of supplying plant nutrients, is also associated with stimulating the activity of many enzymes contributing to the improvement of soil quality.

The data collected in Table 6 show that along with the increase in the dose of the organic form of fertilizer in individual fertilization facilities, the level of the potential biochemical fertility index of the soil also increased. Moreover, the highest index values due to the applied fertilization were obtained for the fertilization objects Pg1 + N and Pg5 + N (respectively 50.03 and 52.91).

The best dynamics of the potential biochemical soil fertility index was obtained for the medium late variety, amounting to 34.11, and the weakest - 30.87 for the medium late planting date. Taking into account the interaction of individual fertilizers and cultivars, the best effect of the increase in the level of potential fertility for sandy loam soil was obtained with the application of the soil improver Pg5 + N in the medium late term (60.06).

Table 6. Index of potential biochemical fertility of loamy sanded soil (autumn 2019)

Fertilization objects /A/	Planting dates /B/			Averages for fertilization
	Wczesny	Średnio późny	Późny	
0	10,78	12,13	10,78	11,23
Nom1+N	50,48	48,33	51,27	50,03
Nom5+N	51,70	46,97	60,06	52,91
Averages for dates	31,75	30,87	34,11	32,24

$SSD_{0,05}$ for: using Pg / A / - 5,83; terms / B / - 1,67; B / A – 3,74; A / B –6,81
0 - control object (without improver). Pg1 + N - soil improver 1 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1. Pg5 + N - soil improver 5 t · ha-1 (N - 100, P - 35, K - 125 kg · ha-1), top dressing N - 60 kg · ha-1.

CONCLUSIONS

1. The highest activity of acid and alkaline phosphatase was found in the soil from the control object, and the lowest in the Pg1 + N object.
2. The highest activity of dehydrogenases was found in the Nom1 + N object, and ureases in the Pg5 + N object.
3. The lowest activities of both enzymes were recorded in the control object.
4. The soil where Pg5 + N was applied, on which the plant was planted in the medium late term, was characterized by the highest content of organic carbon and the highest soil biochemical fertility index.

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Streszczenie: *Wpływ zastosowania polepszacza glebowego na bazie odpadowego węgla brunatnego na aktywność enzymatyczną gleby w uprawie mieszańców paulowni (Paulownia Siebold & Zuccarini, 1835)*. Ważnym elementem kontroli kondycji gleby i uprawianych na niej roślin są testy aktywności enzymatycznej matrycy glebowej. Jedną z największych zalet stosowania testów enzymatycznych jest możliwość dokonania oceny, która zawiera w sobie także inne niemierzalne czynniki mające wpływ na stan i kondycję gleby. Diagnozowane zmian aktywności enzymatycznej gleby są bowiem najlepszym parametrem określania procesów biochemicznych w niej zachodzących. W niniejszym artykule opisuje się aktywność enzymatyczną gleb płowych na których uprawiana jest odmiana mieszańcowa paulowni i stosowany jest polepszacz glebowy na bazie odpadowego węgla brunatnego.

Słowa kluczowe: mieszańce paulowni, aktywność enzymatyczna, polepszacz glebowy, odpadowy węgiel brunatny

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