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APPLICATION OF BIOCHEMICAL INDEX TO DEFINE SOIL FERTILITY DEPENDING ON VARIED ORGANIC AND MINERAL FERTILIZATION

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ABSTRACT

In order to compare the effect of organic and mineral fertilization of various soil types, long-term fertilization experiments frequently use indices, being a function of various parameters considered simultaneously; the content of organic carbon, microbiological parameters and soil enzymatic activity. The aim of the present research was to define soil fertility based on the values of *Biochemical Soil Fertility Index* (B). The index has been formulated based on the research results obtained: enzymatic activity, content of organic carbon and total nitrogen. The research material was sampled from topsoil of a many-year experiment which included varied organic and mineral fertilization, established on typical lessive soil. Soil samples were collected in 1998 from winter wheat stand, four times over the vegetation period. The following enzymes were determined: dehydrogenases, alkaline phosphatases, proteases and amylases. Additionally C_{org} and N_{total} and pH in 1 M KCl were determined. The enzymatic activity of soil and values of the physical and chemical properties researched varied significantly depending on the organic and mineral fertilization applied. Usually the values of the parameters were lowest in soil samples from control objects and those fertilized with mineral fertilizers only. However their greatest values were recorded in soil sampled from plots fertilized with: Manure + NPK + Ca and Manure + NPK + Ca + Mg. The highest (B) index value was shown for soil sampled from objects of full organic and mineral fertilization, while its lowest value – soil sampled from control objects and fertilized with Manure + NPK.

Key words: soil fertility, soil fertility index, organic and mineral fertilization, soil enzymes, dehydrogenases, phosphomonoesterases, amylases, proteases.

INTRODUCTION

Fertilization applied in various forms and doses can have a various effect on soil properties, and especially its bioactivity [22], namely all transformations of compounds and energy conversions. Mineral and organic fertilization stimulates plant and microorganisms development and affects soil enzymes activity [15]. The effect of fertilization on soil enzymes depends on the enzyme character, soil type, form of the fertilizer and time of its application [10].

Research into bioactivity and soil fertility shows that many-year application of unbalanced mineral fertilization frequently results in unfavourable changes in chemical soil properties which are mostly responsible for partial soil degradation, which calls for a necessity of a constant monitoring of soil fertility, using not only common chemical indices but also the biological factor [22].

The results presented both in the domestic and world literature on estimating the soil bioactivity suggest a use of such indices which are based on a total microflora abundance or the activity and abundance of respective physiological groups [8], informing of the intensity of biochemical processes. The intensity can be measured with the content of products of the activity of microorganisms as well as soil enzymatic activity [15].

In order to compare the effect of various organic and mineral fertilization variants on bioactivity of various soil types, especially long-term fertilization experiments, indices are frequently used which are a function of the parameters considered simultaneously. They can be formulated based on the content of organic carbon, microbiological parameters and soil enzymatic activity. The results obtained by some authors show that researching the biological soil-fertility indices in many-year experiments is justifiable because permanent biological changes in soil are most frequently seen only after many-year reaction of the agronomic practices applied [18,19].

The present working hypothesis assumes that many-year varied organic and mineral fertilization can significantly affect both changes in the enzymatic activity and other soil properties. The research aimed at:

- determining the activity of selected enzymes depending on the varied organic and mineral fertilization;
- defining the content of organic carbon, total nitrogen, soil reaction due to a varied many-year fertilization;
- formulating the biochemical fertility index and calculating its value based on the enzymatic activity and the content of organic carbon and total nitrogen;
- searching for the correlation between the enzymatic activity of soil, its fertility index and the other physical and chemical properties of the soil studied.

MATERIAL AND METHODS

The soil material was sampled in 1998 during a successive crop rotation realized in a static single-factor fertilization experiment set up in 1948 at the Mochełek Experiment Station of the Faculty of Agriculture, University of Technology and Agriculture in Bydgoszcz. All the Station soils represent typical lessive soils. For the horizon of the soil researched four levels are defined: Ap, Eet, Bt, C of light loamy very fine sand granulation in the higher-up horizons and medium loam in the Bt horizon and light loam in the C horizon. A share of silt and clay in the arable-and-humus horizon accounts for about 15%. As far as the agricultural applicability is concerned, they represent a good rye complex.

Soil was sampled from four fertilization objects, distributed in the systematic design in five blocks constituting replications:

the following fertilization combinations constituted objects:	explanations for the abbreviations used:
1. No fertilization	Control
2. Straw 5 $t \cdot ha^{-1}$ + NPK	Straw + NPK
3. NPK + Ca	NPK + Ca
4. NPK	NPK
5. Manure	Manure
6. Manure + PK	Manure + PK
7. Manure + NK	Manure + NK
8. Manure + NK + Mg	Manure + NK + Mg
9. Manure + NP	Manure + NP

10. Manure + NP + Mg	Manure + NP + Mg
11. Manure + NPK	Manure + NPK
12. Manure + NPK + Mg	Manure + NPK + Mg
13. Manure + NPK + Ca	Manure + NPK + Ca
14. Manure + NPK + Ca + Mg	Manure + NPK + Ca + Mg

Soil was treated with the following fertilizers: ammonium nitrate, powdery monosuperphosphate or triple granulated, potassium salt – high grade, magnesium sulphate and carbonate lime. Manure at the dose of 50 $t \cdot ha^{-1}$ was applied prior to winter ploughing for sugar beet. Straw at the dose of 5 $t \cdot ha^{-1}$ was applied during post-harvest cultivation also for sugar beet.

Soil was sampled for analysis from the topsoil (5-25 cm) four times (April 4 – date 1, May 12 – date 2, June 25 – date 3 and August 15 – date 4) in the winter wheat vegetation period in 1998, namely in the 50th year of the experiment.

The area on which the experiment was located shows low precipitation. The annual precipitation mean is only 432 mm. In 1998 precipitation was, however, considerably higher than in earlier years and exceeded 600 mm. The highest precipitation is recorded in summer months: July, June and August, accounting for 40% of the annual precipitation, however the lowest – in February and March. The precipitation in respective years, especially months, shows a very high variability and is rarely close to the mean values [12]. The annual mean ground temperature at the depth of 5 cm is 8.8°C. Ground reaches 5°C in the first decade of April, 10°C at the turn of April and May, 15°C – at the turn of the second and third decade of May. In the second decade of July the ground temperature for a while exceeded the threshold of 20°C. Temperature and moisture conditions at the Mochełek Experiment Station over the soil sampling are presented in Table 1.

Table 1. Weather conditions at the Mochełek Experiment Station over the soil sampling months in 1998 (per decade)

Month	Decade	Soil temperature (°C) at the depth of		Precipitation, mm
		5 cm	10 cm	
April	I	7.0	6.4	8.7
	II	7.7	7.1	9.9
	III	13.1	12.0	2.5
May	I	14.4	13.2	33.3
	II	16.4	15.2	0.10
	III	16.6	15.3	13.0
June	I	21.7	20.2	24.3
	II	17.0	16.5	46.2
	III	19.6	18.3	24.2
August	I	19.1	18.4	19.3
	II	18.9	18.1	6.4
	III	14.6	14.5	40.1

Soil samples were dried at the room temperature and then sieved with meshes of 1 mm in diameter. Having been sieved, the soil samples were kept in plastic containers at the temperature of about 18°C and constituted the initial material for the analysis. Respective laboratory analyses were carried out in four replications.

The activity of the following soil enzymes was determined:

- dehydrogenases – colorimetrically according to Thalmann [29] with 2,3,5-triphenyltetrazolium chloride /TTC/ acting as the acceptor;
- alkaline phosphatase in pH 8.5 – colorimetrically following Tabatabai and Bremner [27] using p-nitrophenol phosphate sodium salt as substrate;
- proteolytic enzymes – colorimetrically based on the method developed by Beck [2] which involves determining the amount of amino acids due to enzymatic hydrolysis of gelatine. The method involves determining spectrophotometrically the intensity of colourful complexes which are developed in the solution following the reaction of amino acids with copper;

- amylolytic enzymes – nephelometrically following the method developed by Beck [2] which involves the measurement of the amount of starch which has not been decomposed, having been precipitated by ethanol before.

Also some chemical soil properties were determined:

- organic carbon - with Tiurin method [20],
- total nitrogen - with the distillation method using Büchi [20] distillation apparatus
- pH in 1 M KCl - with the electrometric method, maintaining the weight ratio of soil : solution to be 1 : 2.5 [20].

Based on the obtained values of the enzymatic activity and the content of N_{total} and C_{org} *Biochemical Soil Fertility Index (B)* (following the authors) has been formulated and calculated:

$$B = C_{org} + N_{total} + DHA + F_{al} + Prot + Amyl$$

where:
C_{org} – organic carbon content, %
N_{total} – total nitrogen content, %
DHA – dehydrogenases activity, $cm^3\ H_2 \cdot kg^{-1} \cdot 24h^{-1}$
F_{al} – alkaline phosphatase activity, mmol $PNP \cdot kg^{-1} \cdot h^{-1}$
Prot – proteases activity, mmol $N\text{-}NH_4^+ \cdot kg^{-1} \cdot h^{-1}$
Amyl – amylases activity, mg of decomposed $starch \cdot h^{-1}$

The choice of such parameters for the formulation of B index was made based on their high values of correlation coefficients with the other features of the soil researched, which shows their close relationship with the transformation of the main components of organic matter of soil, namely carbon and nitrogen, under the conditions of the field experiment at Mochełek.

In order to classify the respective soil samples to evaluate soil fertility, the values of the biochemical fertility index were divided into the following ranges (following the authors):

- 3-4 – low fertility,
- 4-5 – average fertility,
- 5-6 – high fertility,
- 6-7 – very high fertility.

RESULTS AND DISCUSSION

1. Chemical properties of the soil researched

The variance analysis of the results obtained showed significant differences in the content of C_{org} and N_{total} depending on the organic and mineral fertilization (Table 2). The highest content of these components of organic matter was recorded in soil sampled from objects with full organic and mineral fertilization with Mg and lime added (objects 13 and 14). The lowest contents of both components were usually determined in soil samples from control objects fertilized only with NPK or also with NPK + Ca. An especially low content of total nitrogen was observed in soil sampled from the object fertilized with Manure + PK which amounted to 0.40 $g \cdot kg^{-1}$ only. Most soil samples researched showed a very acidic reaction (pH to 4.5), only soil samples from objects with full organic and mineral fertilization (13 and 14) showed a slight acid reaction. pH values obtained in the present research show that only a systematic combined application of organic and mineral fertilization with liming can prevent the soil from unfavourable chemical changes.

Table 2. Selected physical and chemical properties of the soil researched

Fertilization objects	Organic carbon	Total nitrogen	pH_{KCl}
	$g \cdot kg^{-1}$		
1. Control	4.68	0.42	4.20
2. Straw + NPK	4.94	0.45	3.56
3. NPK + Ca	4.83	0.42	4.00
4. NPK	4.87	0.43	3.60
5. Manure	5.07	0.47	3.75
6. Manure + PK	4.77	0.40	3.75
7. Manure + NK	5.37	0.53	3.45
8. Manure + NK + Mg	5.45	0.43	3.40
9. Manure + NP	5.50	0.50	3.50
10. Manure + NP + Mg	5.37	0.54	3.73
11. Manure + NPK	5.37	0.52	3.45
12. Manure + NPK + Mg	5.33	0.52	3.35
13. Manure + NPK + Ca	6.45	0.58	5.92
14. Manure + NPK + Ca + Mg	6.74	0.68	5.83
Mean	5.34	0.49	–
$LSD_{0.05}$	0.233	0.128	

2. Enzymatic activity of soil

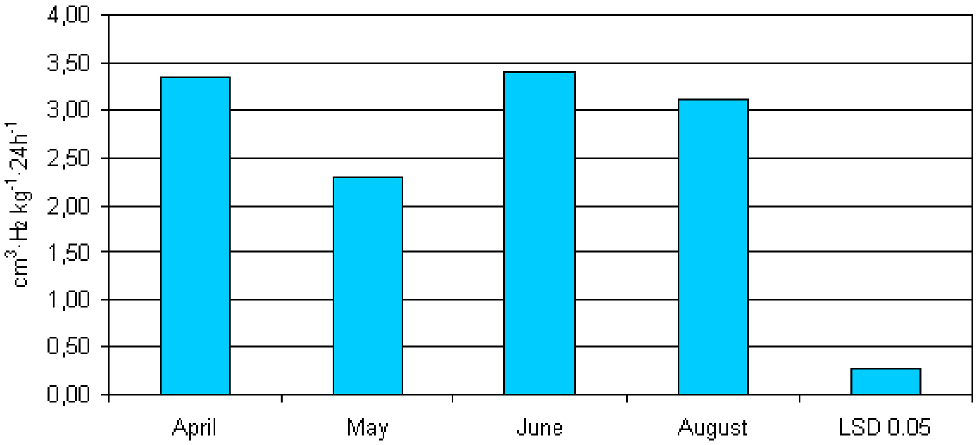
Based on the variance analysis of the results obtained, it was noted that varied fertilization and soil sampling dates significantly affected the enzymatic activity of the soil sampled (Table 3, Figs. 1, 2). A varied organic and mineral fertilization of soil applied for many years in the Mochełek experiment significantly affected the soil enzymatic activity. The activity of dehydrogenases determined in the lessive soil researched was relatively low as compared with literary reports [4,10]. However full organic and mineral fertilization with lime (object 14) significantly increased their activity, which amounted to 4.47 cm^3 $H_2 \cdot kg^{-1}$ of dry matter of soil$\cdot 24h^{-1}$. Significantly lowest activity of the enzymes group researched was recorded in soil sampled from control plots and those fertilized with Straw + NPK.

Table 3. Enzymatic activity of the soil researched depending on varied organic and mineral fertilization

Fertilization objects	Dehydrogenases, cm^3 $H_2 \cdot kg^{-1}$ of dry matter of soil$\cdot 24h^{-1}$	Alkaline phosphatase, mmol PNP$\cdot kg^{-1}$ of dry matter of soil$\cdot h^{-1}$	Proteases, mmol $NH_4^+ \cdot kg^{-1}$ of dry matter of soil$\cdot h^{-1}$	Amylases, mg of decomposed starch$\cdot g^{-1}$ of dry matter of soil$\cdot h^{-1}$
1. Control	2.31	0.24	0.25	0.18
2. Straw + NPK	2.46	0.21	0.24	0.16
3. NPK + Ca	2.83	0.26	0.27	0.17
4. NPK	2.76	0.20	0.21	0.15
5. Manure	4.32	0.22	0.26	0.19
6. Manure + PK	2.98	0.20	0.21	0.19
7. Manure + NK	3.28	0.19	0.23	0.19
8. Manure + NK + Mg	4.03	0.23	0.28	0.20
9. Manure + NP	2.68	0.21	0.24	0.18
10. Manure + NP + Mg	3.21	0.24	0.31	0.20
11. Manure+ NPK	2.01	0.20	0.25	0.20
12. Manure + NPK + Mg	2.61	0.23	0.35	0.21
13. Manure + NPK + Ca	2.91	0.30	0.41	0.23
14. Manure + NPK + Ca + Mg	4.47	0.31	0.44	0.24
Mean	3.06	0.23	0.28	0.19
$LSD_{0.05}$	0.007	0.027	0.035	0.005

Fig. 1. Activity of dehydrogenases (a) and alkaline phosphatases (b) in soil researched depending on the soil sampling date

a) Dehydrogenases



b) Alkaline phosphatase

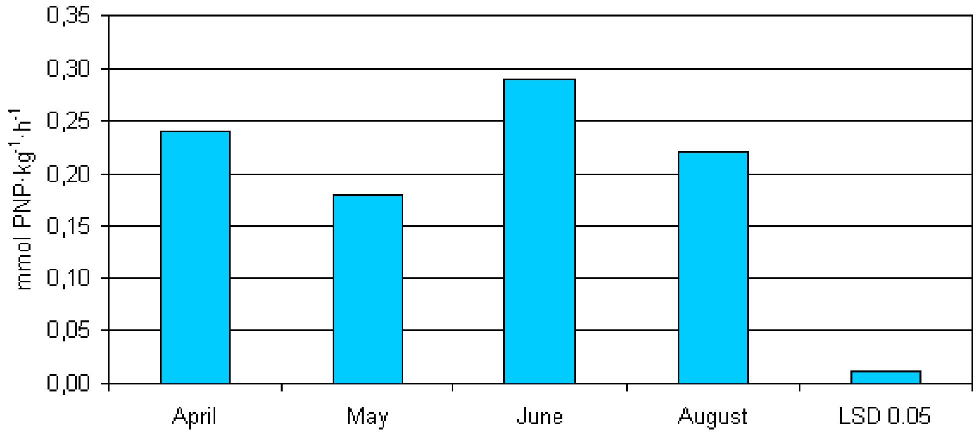
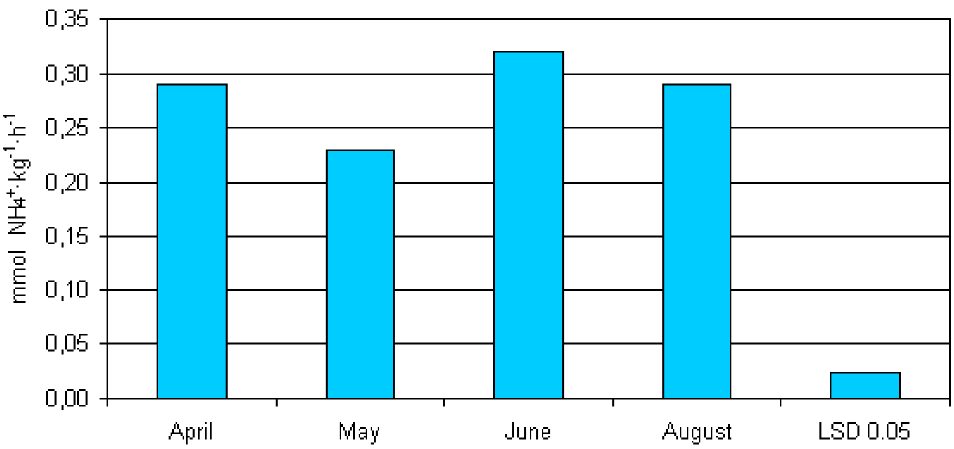
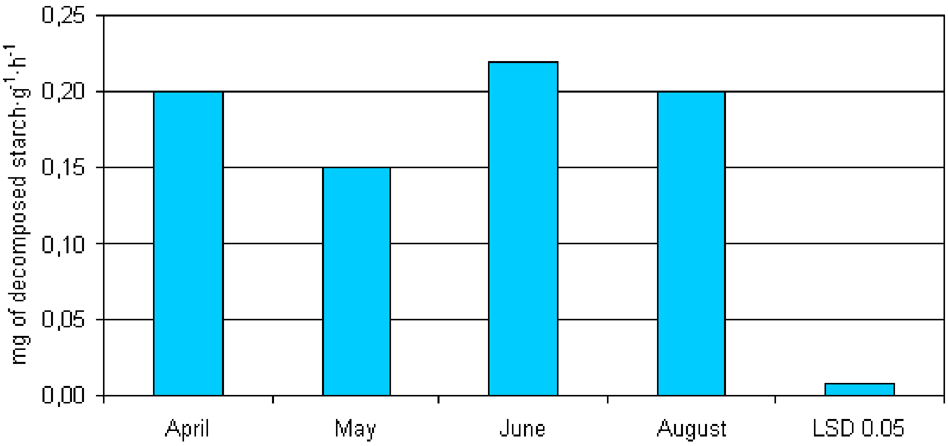


Fig. 2. Activity of proteases (a) and alkaline amylases (b) in soil researched depending on the soil sampling date

a) Proteases



b) Amylases



The research also showed an increased activity of dehydrogenases in soil from the object fertilized with Manure: 4.32 cm^3 $H_2 \cdot kg^{-1}$ of dry matter of soil·$24h^{-1}$. A positive effect of manure on the enzymatic activity is confirmed by numerous research [4,6,19]. Comparing the activity of dehydrogenases in soil from objects Manure + PK and Manure + NPK, one can see that nitrogen fertilization decreased their activity. High N doses tend to increase the inhibition of enzymatic proteins [7]. Comparing the activity of dehydrogenases in soil obtained from the following objects: (Manure + NK) and (Manure + NPK) and (Manure + NK + Mg) and (Manure + NPK + Mg), it was observed that phosphorus fertilization at 44 kg $P \cdot ha^{-1}$ when combined with other factors could have also led to its decrease. Similar trends in changes in the activities of the enzymes analyzed were recorded by Šlimek et al. [24].

Analyzing data contained in Table 3 one can observe that the activity of alkaline phosphatase, similarly to dehydrogenases, was highest in soil sampled from objects with a full organic and mineral fertilization when adding magnesium and lime. Its values ranged from 0.30 to 0.31 mmol $PNP \cdot kg^{-1}$ of dry matter of soil·h^{-1}. A considerably high activity of the enzyme marked was observed also in soil sampled from the objects on which magnesium was added (objects 8, 10 and 12), and also from object NPK + Ca. Soil fertilized for many years only with NPK and Manure + PK and Manure + NK showed the lowest activity of alkaline phosphatase as compared with the activities observed in soil of the other objects analyzed. Analyzing the effect of respective fertilizers applied in the experiment, one shall observe that magnesium and soil liming significantly increased the activity of phosphatase in the soil samples studied, however nitrogen, phosphorus and potassium fertilizer did not show any significant effect on the activity of the enzyme studied.

The activity of the other hydrolytic enzymes, namely amylases and proteases was higher in soil sampled from objects with a full organic and mineral fertilization adding Mg and lime (objects 13 and 14). The lowest and inconsiderably varied values of proteases activity were obtained for soil samples from objects fertilized with NPK, Manure + PK, while amylases from plots which were fertilized with Straw + NPK, NPK + Ca and NPK. Comparing the activity of proteases and amylases in soil sampled from objects fertilized with NPK and Manure + NPK did not show a significant effect of manure on the proteolytic activity, however it was observed that the activity of amylases increased significantly. Literature reports show that both increasing [8] and decreasing [24] the activity of proteolytic enzymes is affected by nitrogen fertilization.

Liming increased the activity of proteases and amylases in soil; especially significant difference was recorded for the activity of proteases in soil sampled from objects fertilized with Manure + NPK and Manure + NPK + Ca. A favourable effect of liming on the activity of many soil enzymes is well known [1,5]; calcium stabilizes some proteolytic enzymes and protects them against autolysis [31]. Enzymatic activity of the soil studied changed over winter wheat vegetation period. The highest activity of dehydrogenases was recorded in soil sampled at the third date and amounted to 3.43 cm^3 $H_2 \cdot kg^{-1}$ of dry matter of soil·$24h^{-1}$, however the lowest in soil sampled at the second date: 2.31 cm^3 $H_2 \cdot kg^{-1}$ of dry matter·$24h^{-1}$. A lowered activity of dehydrogenases in soil for that sampling date could have been due to a low precipitation recorded over that period, as compared with their level obtained for the fourth date. A low soil moisture can lead to a partial reduction in the microbiological activity and related activity of dehydrogenases [26].

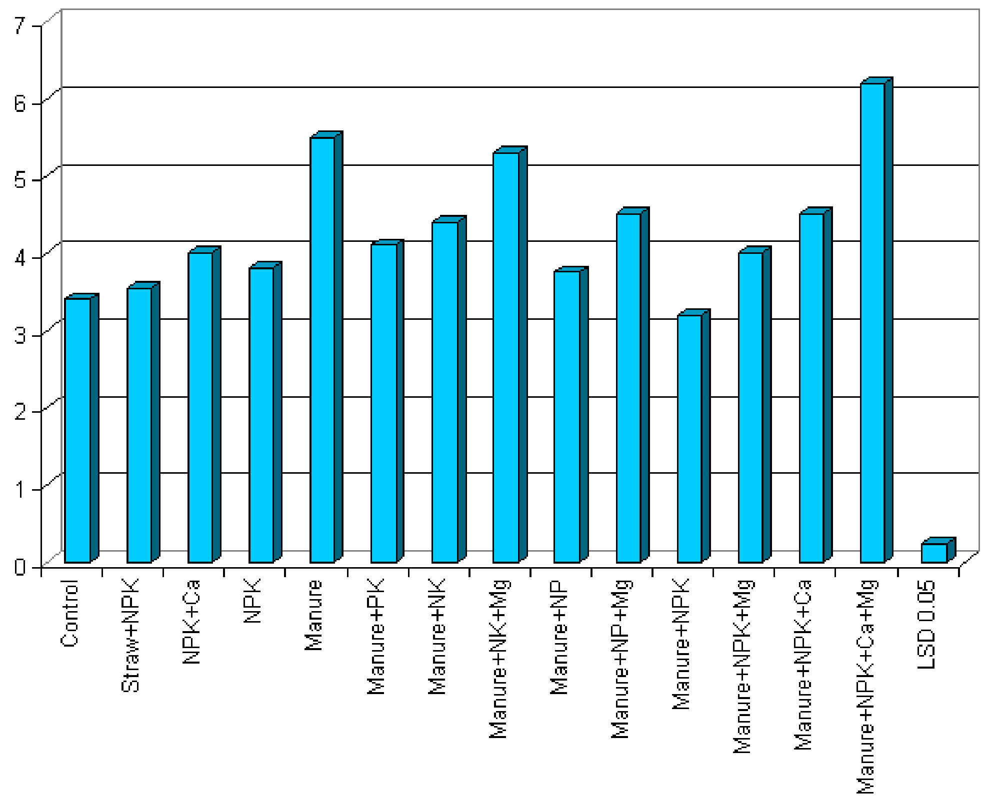
The highest activity of amylolytic and proteolytic enzymes and alkaline phosphatase was obtained in soil sampled at the third date (June), however the lowest values of these activities were recorded in soil sampled in May. Frequently the activity of amylases increases along with a plant vegetative development. In earlier research maximum activities of these enzymes were reported by Ross [21] in soil researched in the second half of summer. A low activity of proteases obtained in soil sampled at the second and fourth date (May and August) of the present research could have been due to a low precipitation over these months. Moisture is of paramount importance for proteins mineralization processes as well as for other metabolic processes [13]. There was observed a close relation between the degree of protein hydrolysis and soil moisture [30].

The recorded seasonal changes in hydrolytic enzymes in lessive soil from Mochełek were similar to the activity of dehydrogenases, which suggests that the enzymes were mostly of microbiological origin.

3. Biochemical soil fertility index

To compare the effect of a varied organic-and-mineral fertilization on the soil bioactivity in many-year fertilization experiments it can be useful to involve indices which would define the relationship between the activity and the soil fertility [25]. Myśków et al. [19], when formulating such indices prefer the use of enzymatic activity, claiming that, contrary to defining the count of selected groups of microorganisms, defining the activity of enzymes is more convenient and easier to carry out serial analyses.

Fig. 3. Values of *Biochemical Soil Fertility Index* (B) depending on varied organic and mineral fertilization



Based on the analytic values of the enzymatic activity in the present research and the content of organic carbon and total nitrogen in lessive soil at Mochełek the *Biochemical Index of Soil Fertility* was proposed. The values of the index, following the formula given in the methodology (Fig. 3), were calculated from mean values of respective parameters obtained for soil for respective soil sampling dates, ranging from 3.25 in soil from the object Manure + NPK to 6.20 in soil from the objects Manure + NPK + Ca + Mg. A low value of the fertility index was noted for soil from control plots, Straw + NPK, NPK, Manure + NP and Manure + NPK and Manure + NPK + Ca. However, mean fertility according to the index calculated, was recorded for soil from the following objects NPK + Ca, Manure + PK, Manure + NK, Manure + NP + Mg and Manure + NPK + Ca + Mg. Only soil sampled from two objects, namely those fertilized with Manure and Manure + NK + Mg represented a high-fertility class. The highest fertility was recorded for soil with a full organic-and-mineral fertilization: Manure + NPK + Ca + Mg. It represented very high fertility soils (B index value exceeded 6).

4. Correlation between the enzymatic activity, biochemical fertility index and other characteristics of the soil researched

For the results of the enzymatic activity and the other characteristics studied correlation analysis was carried out. The correlation coefficients calculated are presented in Table 4. The content of organic carbon, total nitrogen and the values of pH_{KCl} were significantly and positively correlated with the activity of all the enzymes studied. However the highest values of correlation coefficients (0.67*- 0.74*) were obtained for the values of these characteristics and proteolytic activity of the soil researched. High values of Pearson coefficient between the amount of C_{org} and N_{total} and the enzymatic activity show that it is closely related with the level of organic matter [9]. The soil organic matter protecting enzymes from unfavourable factors prolongs their period of activity in soil [14]. Numerous studies recorded significant correlation coefficients between the content of C_{org} and dehydrogenases activity in soils with various crop rotation (monoculture, traditional crop rotation, soil), treated with organic and mineral fertilizer [3,17,28]. Similarly proteolytic and amylolytic soil activity is frequently positively correlated with the content of C_{org} and N_{total} [3,16,23].

The activity of most soil enzymes was significantly correlated with soil pH, which was confirmed by correlation coefficients in the present research (Table 4). Higher values of correlation coefficients for the relations between pH and alkaline phosphatase activity were reported by Stefanic et al. [25] (r = 0.86*-0.94*), Acosta-Martinez and Tabatabai [1] and for pH and proteases activity – by Gostkowska et al. [11].

Table 4. Linear correlation coefficients between the characteristics of the soil studied

Specification	DHA activity of soil dehydrogenases	$F_{al.}$ - activity of alkaline phosphatase	Proteolytic activity	Amylolytic activity	B
C_{org}	0.36*	0.47*	0.74*	0.60*	0.97*
N_{total}	0.36*	0.40*	0.69*	0.57*	0.89*
pH_{KCl}	0.33	0.57*	0.67*	0.42	0.74*
DHA	–	0.24	0.31*	0.22	–
$F_{al.}$ activity of alkaline phosphatase	0.24	–	0.80*	0.67*	–
Proteolytic activity	0.31*	0.80*	–	0.81*	–
Amylolytic activity	0.22	0.67*	0.81*	–	–

The applicability of indices to evaluate the fertility and yielding potential depends also on their correlation relationships with other soil characteristics, including the content of C_{org}, N_{total} or other macro- and microelements, soil pH and crop yields. For the index formulated and calculated in the present research there were obtained high coefficients, especially with the content of C_{org} (r = 0.97*) and N_{total} (r = 0.89*), which shows an adequate selection of characteristics used in the calculation process.

CONCLUSIONS

1. No soil fertilization, fertilization only with mineral fertilizers or straw fertilization significantly decreased the enzymatic activity in most soil samples from these objects and the content of organic carbon and total nitrogen.
2. The highest values of the enzymatic activity obtained and the soil fertility index in soil samples with full organic-and-mineral fertilization accompanied by liming and magnesium added show that only in such conditions it obtains the highest bio-activity, enhancing its fertility.
3. A high variability in the enzymatic activity observed in soil sampled throughout the research period shows a high applicability of enzymes studies as the index of intensity of changes in soil due to soil sampling date or plant development phase.
4. The obtained significant and positive correlation coefficients between the enzymatic activity and biochemical soil fertility index and the other its characteristics show their close interaction in biochemical changes in the soil researched. Besides they confirm the applicability of the formulated and calculated index to evaluate the soil fertility under the experimental conditions.
5. The soil fertility ranges based on the values of biochemical index (B) allowed for classifying the samples researched from most fertilization objects as a soil of low and medium fertility. Only full organic-and-mineral fertilization, including liming and adding magnesium (object 9 and 14) and fertilizing with manure enhanced the physical and chemical soil properties, which classified the soil as high and very high fertility class.

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