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EFFECT OF SPRING BARLEY NITROGEN FERTILISATION ON THE CHANGES IN THE CONTENT OF PHOSPHORUS AND THE ACTIVITY OF ALKALINE AND ACID PHOSPHATASE IN SOIL

WPŁYW NAWOŻENIA AZOTEM JĘCZMIENIA JAREGO NA ZMIANY ZAWARTOŚCI FOSFORU I AKTYWNOŚCI FOSFATAZY ALKALICZNEJ I KWAŚNEJ W GLEBIE

Abstract: The aim of the present paper was to determine changes in the content of available phosphorus and the activity of alkaline and acid phosphatase as a result of nitrogen fertilisation at the following rates: 0, 30, 60, 90, 120, 150 kgN $\cdot$ ha^{-1} and mineral fertilisation (P, K, Mg, Ca and S) in soil under spring barley. The soil was sampled in June and October 2008 from a field experiment set up in the area of the Agricultural Experiment Station at Grabow on the Vistula, by the Institute of Soil Science and Plant Cultivation (IUNG) in Pulawy. There was found a significant effect of nitrogen fertilisation on the content of available phosphorus in the soil. High nitrogen rates resulted in a decrease in the content of that macroelement as well as a decrease in the activity of alkaline phosphatase both in the soil sampled in June and in October. The activity of acid phosphatase, due to increasing nitrogen rates, was increasing. Complete mineral fertilisation (P, K, Mg, S and Ca) fully satisfied the nutrition requirements of spring barley. There was identified a seasonal variation in the content of phosphorus and the activity of alkaline and acid phosphatase in the Luvisol.

Keywords: nitrogen, mineral fertilization, pH, available phosphorus, alkaline and acid phosphatases, soil

Including into the domestic law of the Regulation of Minister of Environment [DzU of 2003 No 4, item 44] [Regulation 2002] [1], pursuant to Nitrates Directive [Directive 91/676/EEC] [2], was the first step to reduce a negative effect on the environment. Thus there was recognised the effect of agroecosystems, especially the application of nitrogen and phosphorus, on the deterioration of the quality of waters, causing *eg* eutrophication. Unfortunately agricultural practise points to the fact that nitrogen fertilisation is preferred, as the basic yield-forming factor which, when provided in excessive amounts, results in soil and water contamination with nitrates. The supply of plants with nutrients

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in the adequate proportions is among the basic conditions of producing optimal crop yields [3, 4].

Chemicals uptaken from soil with yields are mostly supplemented by mineral fertilisation which, at the same time, modifies chemical and biochemical soil properties [5].

An excess of one of the minerals can cause or intensify the deficit of others, even when exposed to their optimum content in soil. This phenomenon is referred to as the antagonism of ions. A lack of balance between high rates of nitrogen, being the main yield-forming element, and the insufficient level of fertilisation with the other nutrients can lead to soil degradation and thus to a decrease in yields.

The aim of the paper was to determine the effect of fertilisation with increasing nitrogen rates and nutrients (P, K, Mg, Ca and S) on the content of available phosphorus and the activity of alkaline and acid phosphatase in soil under spring barley.

Material and methods

The present research was based on a many-year static field experiment, set up by the Department of Plant Nutrition and Fertilization of the *Institute of Soil Science and Plant Cultivation* (IUNG) in Pulawy. Strict experiments were performed in June and October 2008 in a long-term permanent experimental field located in the area of the Agricultural Experiment Station at Grabow on the Vistula, the Mazowieckie province, the zwolenski county, the commune of Przyleki. The location of the station is determined by latitude ($51^o21'8''$ N) and longitude ($21^o40'8''$ E). Lowland climate, of moderate latitudes. The soils the Agricultural Experiment Station at Grabow, represent Haplic Luvisols, of a very good rye complex soil. The experiment was performed in a four-year crop rotation: winter wheat + intercrop, corn for grain, spring barley, winter rape.

It was a two-factor experiment, in the randomised block design, in two reps. The first factor involved P, K, Mg, Ca and S fertilisation at six levels: 1 – (P, K, Mg, Ca and S), 2 – (K, Mg, Ca and S), 3 – (P, Mg, Ca and S), 4 – (P, K, Ca and S), 5 – (P, K, Mg, Ca), 6 – (P, K, Mg, S). The second factor involved nitrogen fertilisation at the following rates: 0, 30, 60, 90, 120, 150 $kgN \cdot ha^{-1}$.

The following fertiliser forms were applied: for the treatments with S there were used phosphorus and potassium fertilisers containing sulphur: single superphosphate and potassium sulphate, for the treatment without sulphur there were used phosphorus and potassium fertilisers which do not include sulphur: triple superphosphate and high-percentage potassium salt, for the treatment with Ca and Mg there was applied dolomite containing 30 % CaO and 10 % Mg, in the plots without Mg, lime was used at the amount of 200 $kgCaO \cdot ha^{-1}$, while in the case of Ca deficit, magnesium sulphate was supplied at the rate of 70 $kgMgO \cdot ha^{-1}$. The rates of minerals applied in the experiment were as follows: 80 $kgP_2O_5 \cdot ha^{-1}$, $140K_2O \cdot ha^{-1}$, $70MgO \cdot ha^{-1}$, $200CaO \cdot ha^{-1}$. The S rate is a result of the sulphur amount introduced with adequate rates of P, K, Mg.

The soil was sampled twice: in June and in October 2008 under spring barley.

In the adequately prepared material, the following were determined:

– the content of available phosphorus (P_{E-R}) with the Egner-Riehm method – DL [6];

– the activity of alkaline (AlP) and acid (AcP) phosphatase with the Tabatabai, Bremner method [7];

– pH in H_2O [6].

The results were exposed to the analysis of variance and the significance of differences between means was verified with the Tukey test at the confidence level of p = 0.05. The calculations involved the use of FR-ANALWAR software based on Microsoft Excel. To identify the potential correlations between soil parameters the statistical analysis of the results was made applying the Statistica software.

Results and discussion

The mineral fertilisation applied in the experiment resulted in slight changes in the active soil acidity. The values of pH_{H_2O} ranged from 5.2 to 5.9 in soil depending on fertilisation (Table 1). Drawing on the pH_{H_2O} values recorded, the soil can be classified as acid.

Table 1

Exchangeable acidity of the Luvisol investigated, depending on differentiated increasing nitrogen rates and mineral fertilization (P, K, Mg, Ca, S)

Nitrogen $[kg \cdot ha^{-1}]$	Mineral fertilization					
	P K Mg Ca S	K Mg Ca S	P Mg Ca S	P K Ca S	P K Mg S	P K Mg Ca
0	5.8	5.9	5.7	5.9	5.6	5.9
30	5.8	5.8	5.7	5.9	5.6	5.9
60	5.7	5.7	5.8	5.8	5.5	5.8
90	5.8	5.8	5.7	5.8	5.5	5.9
120	5.7	5.7	5.6	5.5	5.3	5.8
150	5.6	5.7	5.5	5.3	5.1	5.7

The content of available phosphorus in the Luvisol investigated was 57.74 $mgP \cdot kg^{-1}$, which, according to the criteria provided for in PN-R-04023 [1996] [8], classifies it as class III, of an average content of $P_{E\text{-}R}$. Fotyma et al [9] claim that the optimal content of available phosphorus (determined with the Egner-Riehm method) should be 105–108 $mgP \cdot kg^{-1}$. There was found a significant effect of a varied mineral fertilisation on the content of phosphorus in soil under spring barley. The highest content of that nutrient was reported in the soil sampled both in June (59.0 $mgP \cdot kg^{-1}$) and in October (77.0 $mgP \cdot kg^{-1}$) with complete mineral fertilisation (P, K, Mg, Ca, S) (Table 2). As a result of the lack of phosphorus fertilisation (K, Mg, Ca, S), a significant decrease in the content of $P_{E\text{-}R}$ (by about 33 %) was noted, as compared with complete fertilisation. According to Szara et al [10], despite the lack of phosphorus fertilisation and persisting low content of available phosphorus in soil, the plants can uptake considerable amounts of phosphorus from the form which is hardly available. In the soil non-fertilised with Ca (P, K, Mg, S) there also occurred a decrease in $P_{E\text{-}R}$ in the soil sampled in June (51.2 $mgP \cdot kg^{-1}$) and in October (55.9 $mgP \cdot kg^{-1}$) (Table 2). According to Bednarek and

Table 2

Content of available phosphorus [mgP · kg^{-1}] in the Luvisol investigated, depending on differentiated increasing nitrogen rates and mineral fertilization (P, K, Mg, Ca, S)

Mineral fertilization I factor	June							October						
	Nitrogen II factor [kgN · ha^{-1}]													
	0	30	60	90	120	150	Mean	0	30	60	90	120	150	Mean
P K Mg Ca S	76.5	67.0	61.0	58.1	50.5	41.1	59.0	87.8	92.6	81.2	73.8	69.8	56.8	77.0
K Mg Ca S	56.9	50.1	42.9	38.8	33.9	27.2	41.7	58.9	65.8	51.4	44.1	40.0	37.1	49.6
P Mg Ca S	64.7	59.9	55.8	51.1	46.1	39.1	52.8	79.9	88.0	73.2	66.1	57.5	49.6	69.1
P K Ca S	69.9	66.3	59.9	55.3	48.5	42.3	57.0	73.6	67.3	61.9	57.7	51.6	45.3	59.6
P K Mg S	61.2	58.3	52.8	49.9	46.1	39.1	51.2	66.9	69.9	58.7	57.3	43.1	40.0	55.9
P K Mg Ca	66.3	60.8	56.8	50.2	46.1	41.8	53.8	83.9	77.9	68.8	60.0	58.2	48.6	66.2
Mean	65.9	60.4	54.8	50.5	45.3	38.4	52.6	75.1	76.9	65.9	59.8	53.4	46.3	62.9
$LSD_{0.05}$														
I factor	1.178							1.456						
II factor	1.178							1.456						
Interaction														
I/II	2.885							3.567						
II/I	2.885							3.567						

Reszka [11], no liming increases the concentration of soluble and exchangeable ions of Fe and Al which can react with the phosphorus applied, which, as a result, leads to the formation of slightly soluble aluminium phosphates and iron phosphates. In the soil sampled from the treatments without sulphur (combination P, K, Mg, Ca), the content of available phosphorus in June was 53.8 mgP · kg^{-1}, while in October – 66.2 mgP · kg^{-1}. According to Kaczor and Laszcz-Zakorczmenna [4], sulphur deficit in soil can limit the uptake of basic nutrients by plants, including phosphorus, as a result of which the soil from the treatments non-fertilised with sulphur showed a greater richness with the available form of phosphorus.

Nitrogen fertilisation showed a significant effect on the content of available phosphorus in soil. In the soil sampled in June the highest content of the available form of phosphorus (65.9 mgP · kg^{-1}) was reported for the treatments non-fertilised with nitrogen, while in October the highest content of $P_{E\text{-}R}$ was recorded in the soil from the treatments fertilised with nitrogen at the rate of 30 kgN · ha^{-1} (76.9 mgP · kg^{-1}). Similarly Szymanska et al [12] claim that in the soil sampled from the treatments where for a few years nitrogen fertilisation had been applied, there was observed a decrease in the content of available phosphorus by an average of about 23 %, as compared with the soil from the treatments non-fertilised with nitrogen. The application of nitrogen at the rate of 150 kgN · ha^{-1} resulted in a clear decrease in the content of $P_{E\text{-}R}$ do 38.4 mgP · kg^{-1}. As the content of phosphorus critical for the plants 30 mgP · kg^{-1} soil is assumed. According to Rabikowska and Piszcz [13], many-year application of increasing nitrogen rates (maximum to 210 kgN · ha^{-1}) results in a decrease in soil fertility, an increase in its acidity, a decrease in the share of alkaline cations in the sorption capacity and in a decrease in the content of mobile forms of nutrients, including phosphorus. Most phosphorus is released when pH of soil ranges from 6 to 7.

The research demonstrated a significant effect of the fertilisation applied on the changes in the activity of alkaline and acid phosphatase in Luvisol. The highest AlP activity was noted in the soil sampled from the treatments without phosphorus fertilisation (K, Mg, Ca, S) (0.600 mM pNP/ kg · h in June, 0.777 mM pNP/kg · h in October) (Table 3). A high activity of that enzyme in soils points to an intensive rate of releasing anions $H_2PO_4^-$ and HPO_4^{2-} available to plants from organic bonds of phosphorus. For the plants it is an important source of supply with phosphorus, especially in the combinations non-fertilised with P-combination (K, Mg, Ca, S).

According to Heflik et al [14], Zebrowska et al [15], the reaction to phosphorus deficit in soil involves the production of phosphatases both via plant roots and microorganisms and so the activity of those enzymes is conditioned by the content of soil phosphorus, while Sapek [16] noted a lower efficiency of phosphorus mineralization once the fertilisation with that nutrient was abandoned, which could have been due to a poorer activity of soil microorganisms. As shown by Saggar et al [17], in the soils poorly fertilised with phosphorus the transformations of carbon are less intensive due to low efficiency of the microorganisms biomass.

The lowest activity of alkaline phosphatase (0.324 mM pNP/kg · h in June, 0.417 mM pNP/kg · h in October) was reported in the soil without liming (combination P, K,

Table 3

Activity of alkaline phosphatases [mM pNP/kg · h] in the Luvisol investigated, depending on differentiated increasing nitrogen rates and mineral fertilization (P, K, Mg, Ca, S)

Mineral fertilization I factor	June							October						
	Nitrogen II factor [kgN · ha^{-1}]													
	0	30	60	90	120	150	Mean	0	30	60	90	120	150	Mean
P K Mg Ca S	0.561	0.577	0.620	0.551	0.505	0.432	0.541	0.612	0.640	0.655	0.614	0.583	0.575	0.613
K Mg Ca S	0.596	0.610	0.659	0.630	0.572	0.533	0.600	0.789	0.814	0.832	0.770	0.747	0.710	0.777
P Mg Ca S	0.493	0.534	0.579	0.546	0.509	0.448	0.518	0.547	0.590	0.605	0.551	0.528	0.510	0.555
P K Ca S	0.330	0.392	0.441	0.383	0.358	0.288	0.365	0.452	0.481	0.498	0.445	0.420	0.403	0.450
P K Mg S	0.296	0.350	0.364	0.350	0.316	0.270	0.324	0.423	0.435	0.460	0.422	0.402	0.360	0.417
P K Mg Ca	0.455	0.478	0.507	0.494	0.454	0.416	0.467	0.560	0.586	0.593	0.540	0.509	0.502	0.548
Mean	0.455	0.490	0.528	0.492	0.452	0.398	0.469	0.564	0.591	0.607	0.557	0.531	0.510	0.560
$LSD_{0.05}$														
I factor	0.007							0.007						
II factor	0.007							0.007						
Interaction														
I/II	0.017							0.017						
II/I	0.017							0.017						

Mg, S). In those soils there was reported the lowest soil reaction (Table 1) which controls the activity of that enzyme considerably.

The fertilisation with nitrogen showed a significant effect on the activity of alkaline and acid phosphatase. Increasing nitrogen rates stimulated the activity of acid phosphatase, however, they inhibited the activity of alkaline phosphatase. After the nitrogen application at the rate of 150 kgN · ha^{-1} there was found the highest activity of acid phosphomonoesterase, while the activity of alkaline phosphatase was lowest, both in the soil sampled in summer and in autumn (Table 3). A poorer activity of alkaline phosphatase as a result of the increase in soil acidity is a result of the destruction of hydrogen and ionic bonds in the active enzyme centre so slight pH changes can decrease its activity. Excessively high nitrogen rates can lead to *eg* the accumulation of toxic substances or ammonia intoxicating plants and limiting the development of some groups of microorganisms being one of the sources of enzymes [18].

Based on the values of activity of alkaline and acid phosphatase recorded, there was calculated the ratio of AlP : AcP, referred to as the enzymatic index of the pH level [19]. The values of the AlP : AcP ratio throughout the research ranged from 0.28 to 0.57 (Fig. 1). The soil pH value to be considered adequate for the plant growth and development can be the one under the conditions of which there occurs the right ratio of the activity of AlP : AcP [19]. According to those authors [19], the value of the AlP : AcP ratio lower than 0.50 points to the acid reaction of soil and limiting is recommended. In the soil investigated for which no liming was applied (P, K, Mg, S), the value of the enzymatic index of the pH level was low (0.26–0.29). With the increasing nitrogen rate, the value of the AlP : AcP ratio in the soil decreased, after the application of the highest nitrogen rate (150 kgN · ha^{-1}) the AlP : AcP value was lowest (0.28–0.30). Similarly pH, measured in H_2O, was lowest in the soil fertilised with nitrogen at the rate of 150 kgN · ha^{-1}. Only in the soil sampled in June from the treatments fertilised with complete mineral fertilisation (combination P, K, Mg, Ca, S) and non-fertilised with nitrogen and at the rate of 30 kgN · ha^{-1}, the AlP : AcP value

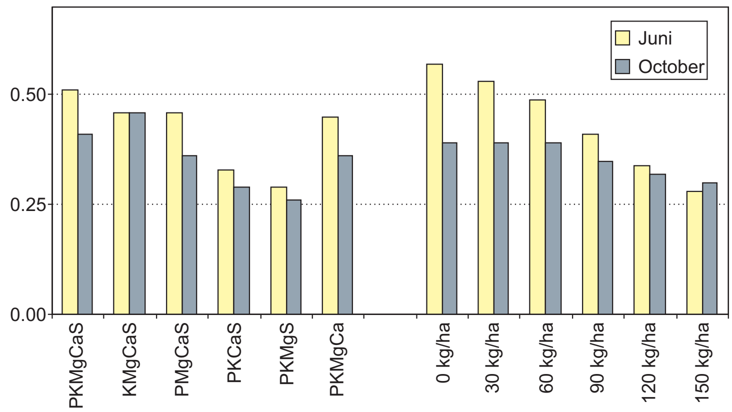

Fig. 1. Ratio of alkaline to acid phosphatase AlP : AcP in the Luvisol investigated, depending on the differentiated increasing nitrogen rates and mineral fertilization (P, K, Mg, Ca, S)

Table 4

Activity of acid phosphatases [mM pNP/kg · h] and the ratio of alkaline to acid phosphatase AlP : AcP in the Luvisol investigated, depending on differentiated increasing nitrogen rates and mineral fertilization (P, K, Mg, Ca, S)

Mineral fertilization I factor	June							October						
	Nitrogen II factor [kgN · ha^{-1}]													
	0	30	60	90	120	150	Mean	0	30	60	90	120	150	Mean
P K Mg Ca S	0.786	0.846	0.974	1.105	1.283	1.322	1.052	1.383	1.409	1.480	1.500	1.557	1.628	1.493
K Mg Ca S	0.973	1.140	1.224	1.388	1.504	1.598	1.304	1.593	1.627	1.664	1.726	1.781	1.807	1.699
P Mg Ca S	0.710	0.878	1.112	1.248	1.338	1.433	1.120	1.403	1.454	1.502	1.538	1.618	1.659	1.529
P K Ca S	0.798	0.882	1.037	1.197	1.289	1.361	1.094	1.443	1.490	1.539	1.595	1.593	1.688	1.558
P K Mg S	0.806	0.910	1.101	1.200	1.310	1.427	1.125	1.511	1.562	1.599	1.640	1.671	1.708	1.615
P K Mg Ca	0.752	0.847	0.977	1.065	1.192	1.366	1.033	1.407	1.457	1.496	1.560	1.616	1.685	1.537
Mean	0.804	0.917	1.071	1.200	1.319	1.417	1.121	1.457	1.499	1.547	1.593	1.639	1.696	1.572
$LSD_{0.05}$														
I factor	0.030							0.023						
II factor	0.030							0.023						
Interaction														
I/II	0.075							n.s.*						
II/I	0.075							n.s.*						

n.s.* – non-significant.

exceeded 0.5. The enzymatic index of the soil pH level can be used as an alternative method to determine the soil reaction and the transformations in it. A lack of organic or natural fertilisation resulted in the soil phosphorus depletion and soil acidification. FYM is a fertiliser alleviating the effects of unbalanced mineral fertilisation. In the soils demonstrating excessively high pH values, FYM can increase their acidity thanks to the formation of organic acids and carbon acid as a result of the hydrolysis and further mineralization of organic compounds.

A higher activity of both phosphatases (alkaline – 16 % higher, acid – 29 % higher) was found in the soil sampled in October, namely after spring barley harvest (Tables 3 and 4), which is connected with a long period of decomposition of organic matter in soil as post-harvest residue is a perfect source of energy for soil microorganisms.

The activity of acid phosphatase was closely connected with the content of phosphorus available to plants. The value of the coefficient of correlation between the activity of AcP and the content of $P_{E\text{-}R}$ in soil was $r = -0.97$, $p < 0.05$ (Table 5). The reports by Wyszolmirska et al [20] suggest that under available phosphorus deficit in the soil there occurs an increase in the activity of intercellular acid phosphatases in the plant tissues (cucumber, two oat cultivars). Similarly the surplus of available forms of phosphorus, which acts as a competition inhibitor, inhibits the synthesis of phosphatases. It is the phenomenon of enzyme repression and thus the inhibition of the activity of a given enzyme by an excess of the final product of the enzymatic activity. Bielinska and Ligeza [21], on the other hand, pointed to a strict correlation between the activity of phosphatases and the content of available phosphorus; a high activity of the phosphatases investigated was connected with the $P_{E\text{-}R}$. content, which was many-fold higher than in the soils of control treatments.

Table 5

Correlation coefficients between the enzymatic activity of soil and available phosphorus contents and pH

Parameters	Available phosphorus	pH
Alkaline phosphatase	n.s.	0.58*
Acid phosphatase	–0.97*	–0.59*

n.s. – non-significant.

There was reported a significant value of the coefficient of correlation between soil pH and the activity of alkaline phosphatase ($r = 0.58$, $p < 0.05$) (Table 5). Similarly Acosta-Martinez and Tabatabai [22] observed a high significant value of the coefficient of correlation ($r = 0.95$*) for the pH and the alkaline phosphomonoesterase activity relationship. A significant, however, negative value of the coefficient of correlation ($r = -0.69$, $p < 0.05$) was reported by those authors for the activity of acid phosphatase and soil pH.

Conclusion

1. Considering the criteria provided for in PN-R-04023, the soil investigated demonstrated an average content of phosphorus available to plants. Nitrogen at the rate

over 30 kgN · h^{-1} resulted in a decrease in the content of available phosphorus in the soil, especially for the treatments non-fertilised with phosphorus.

2. The lack of balance between an insufficient level of fertilisation with P, K, Mg, Ca, S, and increasing nitrogen rates resulted in an unfavourable decrease in the content of phosphorus available in soil.

3. The increasing nitrogen rates resulted in an increase in the activity of acid phosphatase, while the activity of alkaline phosphatase was decreasing.

4. The values of the enzymatic index of the pH level call for soil liming, especially from the treatments fertilised with nitrogen at the rates over 30 kgN · h^{-1}.

5. The activity of alkaline and acid phosphatase was higher in the soil non-fertilised with phosphorus, which points to the participation of those enzymes in the biogeochemical phosphorus cycle.

6. The relationships between the content of available phosphorus in soil and the activity of alkaline and acid phosphatase demonstrate that they are mostly determined by the condition of the soil environment, affected mainly by intensive spring barley fertilisation.

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Abstrakt: Celem pracy było określenie zmian zawartości fosforu przyswajalnego i aktywności fosfatazy alkalicznej i kwaśnej pod wpływem nawożenia azotem w dawkach 0, 30, 60, 90, 120, 150 kgN · ha^{-1} oraz nawożenia mineralnego (P, K, Mg, Ca i S) w glebie spod uprawy jęczmienia jarego. Próbki glebowe pobrano w czerwcu i w październiku 2008 r. z doświadczenia polowego założonego na terenie RZD w Grabowie nad Wisłą, przez IUNG w Puławach. Stwierdzono istotny wpływ nawożenia azotem na zawartość fosforu przyswajalnego w badanej glebie. Duże dawki azotu powodowały zmniejszenie zawartości tego makroskładnika, jak również obniżenie aktywności fosfatazy alkalicznej zarówno w glebie pobranej w czerwcu, jak i październiku. Aktywność fosfatazy kwaśnej pod wpływem wzrastających dawek azotu zwiększała się. Pełne nawożenie mineralne (P, K, Mg, S i Ca) w pełni zaspokoiło potrzeby pokarmowe jęczmienia jarego. Stwierdzono sezonowe zróżnicowanie zawartości fosforu oraz aktywności fosfatazy alkalicznej i kwaśnej w badanej glebie płowej.

Słowa kluczowe: azot, nawożenie mineralne, pH, fosfor przyswajalny, fosfataza alkaliczna i kwaśna, gleba