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# CONTENT OF TOTAL PHOSPHORUS IN SOIL UNDER MAIZE TREATED WITH MINERAL FERTILIZATION AGAINST THE PHOSPHATASE ACTIVITY

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Abstract

The aim of the paper was to determine changes in the content of total phosphorus and its selected forms in soil under maize treated with mineral fertilization. The activity of enzymes participating in transformations of this bioelement was also investigated.

In October 2008, soil was sampled from a field experiment set up at the Agricultural Experimental Station in Grabów nad Wisłą, conducted by the IUNG Institute in Puławy. The experiment involved the application of nitrogen in the form of ammonium nitrate at rates of 0, 50, 100, 150, 200 and 250 kg N $ha^{-1}$ and concomitant P, K, Mg, Ca and S fertilization. The following determinations were made on the soil samples: the content of total phosphorus, phosphorus in organic compounds and available phosphorus as well as the activity of alkaline and acid phosphatases. The analysis of variance showed a significant effect of the experimental factors on changes in the content of total phosphorus and its selected forms. The content of available phosphorus was 55.85 mg $P_{E-R}$ $kg^{-1}$, which classified the soil to class III, that is with a moderate content of this nutrient. The lack of phosphorus fertilization (KMgCaS variant) as well as the use of high nitrogen rates changed the soil phosphorus abundance class from average to low. The soil content of phosphorus in organic compounds was about 25% of the total phosphorus content, but when nitrogen fertilization had been applied at the rates above 100 kg N $ha^{-1}$, $P_{org}$ decreased to about 15%.

The activity of phosphomonoesterases was the highest in soil from the treatments not fertilized with phosphorus (KMgCaS). A close dependence was found between the activity of acid phosphatase and the total phosphorus content or the content of its examined forms. The value of the coefficient of correlation between the AcP activity and the $P_{E-R}$ content in soil was $r$=-0.83, $p$<0.05.

Key words: phosphorus, alkaline and acid phosphatases, mineral fertilization, soil.

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**ZAWARTOŚĆ FOSFORU W GLEBIE SPOD UPRAWY KUKURYDZY NAWOŻONEJ MINERALNIE NA TLE AKTYWNOŚCI FOSFATAZOWEJ**

Abstrakt

Celem pracy było określenie zmian zawartości fosforu ogółem oraz jego wybranych form w glebie spod uprawy kukurydzy nawożonej mineralnie. Zbadano również aktywność enzymów biorących udział w przemianach tego biopierwiastka.

Próbki gleby pobrano w październiku 2008 r. z doświadczenia polowego założonego na terenie RZD w Grabowie nad Wisłą przez IUNG w Puławach. W doświadczeniu zastosowano azot w postaci saletry amonowej w dawkach 0, 50, 100, 150, 200 i 250 kg N $ha^{-1}$ oraz nawożenie P, K, Mg, Ca i S. W próbkach gleby oznaczono zawartość fosforu ogółem, związków organicznych i przyswajalnego oraz aktywność fosfatazy alkalicznej i kwaśnej. Analiza wariancji wykazała istotny wpływ zastosowanych czynników doświadczenia na zmiany zawartości fosforu ogółem i jego wybranych form. Zawartość fosforu przyswajalnego kształtowała się na poziomie 55.85 mg $P_{E\text{-}R}$ $kg^{-1}$, co klasyfikuje glebę do klasy III o średniej zawartości tego składnika pokarmowego. Brak nawożenia fosforem (kombinacja KMgCaS) oraz stosowanie wysokich dawek azotu spowodowało zmianę klasy zasobności gleby w fosfor ze średniej na niską. Zawartość fosforu związków organicznych w glebie stanowiła około 25% całkowitej zawartości fosforu, a przy nawożeniu azotem w dawkach powyżej 100 kg N $ha^{-1}$ spowodowało zmniejszenie $P_{org}$ do ok 15%.

Aktywność badanych fosfomonoesteraz była najwyższa w glebie z obiektów nienawożonych fosforem (KMgCaS). Stwierdzono ścisłą zależność między aktywnością kwaśnej fosfatazy a zawartością fosforu ogółem i jego badanych form. Współczynnik korelacji między aktywnością AcP a zawartością $P_{E\text{-}R}$ w glebie wyniósł $r$=-0.83, $p$<0.05.

Słowa kluczowe: fosfor, fosfataza alkaliczna i kwaśna, nawożenie mineralne, gleba.

# INTRODUCTION

Phosphorus plays a key role in defining the nutrient richness and fertility of soils. It is also a crucial element, indispensable for sustaining proper functions of plants and obtaining high quantities and good quality of yields (GAJ, GRZEBISZ 2003). However, like nitrogen, phosphorus can trigger severe disorders in the natural environment, for example eutrophication of water bodies. *The Regulation of the Minister of the Environment* (2002) based on the Nitrate Directive (91/676/EEC) was the first step towards restraining the negative effect of agriculture on the environment. One of the more serious side-effects of agricultural production in Poland is excessive soil acidification (KACZOR 2002). The main factors responsible for undesirable soil acidification are unbalanced mineral fertilization treatments, especially excessive application of nitrogen fertilizers treated as a key yield-forming factor. Supplying plants with right proportions of nutrients is among the essential conditions assuring optimal crop yields (KACZOR, ŁASZCZ-ZAKORCZMENNA 2009, MURAWSKA, SPYCHAJ-FABISIAK 2009, LEMANOWICZ 2011) However, the amount of a nutrient introduced into soil with fertilizer does not correspond to the amount up taken with the yield. The use of phosphorus by plants does not exceed 40%; the remaining amount is transformed into insoluble forms, unavailable to crops and often leached from soil (Mazur, Mazur 2010). The biogeochemical phosphorus cycle is sensitive to changes induced by agricultural treatments since most mineral fertilizers include this element. This is the reason why agronomic practice often changes the natural phosphorus cycling.

The aim of the paper was to evaluate the content of selected phosphorus forms and the activity of phosphatases in soil under maize treated with mineral fertilization.

## MATERIAL AND METHODS

A long-term field experiment from which soil was sampled had been set up at the Agricultural Experiment Station in Grabów nad Wisłą, by the IUNG Institute of Soil Science and Plant Cultivation in Puławy. The soils at the Experimental Station in Grabów are Haplic Luvisols (PN-R-04033, 1998) classified as a very good rye complex. The experiment was conducted in a four-year rotation cycle: grain maize, spring barley, winter oilseed rape, winter wheat+intercrop.

A two-factorial experiment in a randomised block design was run in two replications. The first factor involved P, K, Mg, Ca and S fertilization at six levels: 1 – P K Mg Ca S, 2 – K Mg Ca S, 3 – P Mg Ca S, 4 – P K Ca S, 5 – P K Mg S, 6 – P K Mg Ca. The second factor consisted of nitrogen fertilization at the following rates: 0, 50, 100, 150, 200, 250 kg N $ha^{-1}$.

The following fertilizer forms were applied: phosphorus and potassium fertilizers containing sulphur such as single superphosphate and potassium sulphate in treatments with sulphur; phosphorus and potassium fertilizers which did not include sulphur such as triple superphosphate and high-percentage potassium salt in treatments without sulphur; dolomite containing 30% CaO and 10% Mg in treatments with Ca and Mg; lime was used at the amount of 200 kg CaO $ha^{-1}$ on plots without Mg, and magnesium sulphate was supplied at the rate of 70 kg MgO $ha^{-1}$ on plots without Ca. The rates of minerals applied in the experiment were as follows: 80 kg $P_2O_5$ $ha^{-1}$, 140 $K_2O$ $ha^{-1}$, 70 MgO $ha^{-1}$, 200 CaO $ha^{-1}$. The S rate is a result of the sulphur amount introduced with adequate rates of P, K, Mg.

Soils samples from fields cropped with maize were collected in October 2008.

The following determinations were made on properly prepared samples of soil material:

– total phosphorus was isolated from soil ($P_{tot}$) with the method by Mehta et al. (1954);

- phosphorus in organic compounds ($P_{org}$) was calculated as the difference between the total P content determined in mineralized samples and the content of inorganic phosphorus determined in non-mineralized samples;
- content of available phosphorus ($P_{E\text{-}R}$) in soil was tested with the Egner-Riehm method – DL (LITYŃSKI et al. 1976);
- the activity of alkaline (AcP) [E.C. 3.1.3.1] and acid phosphatases (AcP) [E.C. 3.1.3.2] in soil was analysed with the TABATABAI and BREMNER method (1969), which enabled the calculation of the AlP:AcP ratio, expressing the right soil reaction (DICK et al. 2000);
- pH in 1M KCl was checked using the potentiometric method.

The data thus achieved underwent an analysis of variance and the significance of differences between the means was defined with Tukey's test at the significance level $p$=0.05. The calculations were made with FR-ANAL-WAR software based on Microsoft Excel. Finally, the results were submitted to an analysis of simple correlation ($p$<0.05), which determined the degree of the dependence between respective features. The correlation analysis was made using Statistica for Windows Pl software.

## RESULTS AND DISCUTION

The exchangeable acidity of soil measured in the humus horizon (Ap) ranged from 5.8 to 6.5 (Table 1), which means that the soil was slightly acid. The mineral fertilization applied exclusively in the experiment differentiated the soil reaction rather weakly. JANOWIAK et al. (2005) showed a decrease in soil pH by 1.7 unit following an application of 180 kg N $ha^{-1}$. According to FILIPEK (2001), nitrogen in the form of ammonium causes acidification of soil solution due to both nitrification and the uptake of $NH_4^+$ cations by the plant root system.

The total phosphorus content in the soil ranged from 0.271 to 0.389 g $P_{tot}$ $kg^{-1}$, depending on the variant of mineral and nitrogen fertilization

Table 1

pH KCl of the soil

| Mineral fertilization | Nitrogen (g $kg^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 |
| P K Mg Ca S | 6.4 | 6.4 | 6.3 | 6.3 | 6.1 | 5.9 |
| K Mg Ca S | 6.5 | 6.4 | 6.4 | 6.3 | 6.1 | 5.8 |
| P Mg Ca S | 6.4 | 6.3 | 6.3 | 6.1 | 6.0 | 5.9 |
| P K Ca S | 6.2 | 6.2 | 6.0 | 6.1 | 5.9 | 5.9 |
| P K Mg S | 6.1 | 6.1 | 6.0 | 5.9 | 5.8 | 5.6 |
| P K Mg Ca | 6.5 | 6.4 | 6.5 | 6.3 | 6.3 | 6.1 |

(Table 2). As reported by VERMA et al. (2005), in the soils fertilized with nitrogen alone, the $P_{tot}$ content was much higher and remained at the level of 0.507 g $P_{tot}$ $kg^{-1}$. The highest $P_{tot}$ content (0.389 g $P_{tot}$ $kg^{-1}$) was found in the soil with complete mineral fertilization (P K Mg Ca S variant) – Table 2. Increasing nitrogen rates decreased the content of total phosphorus in soil.

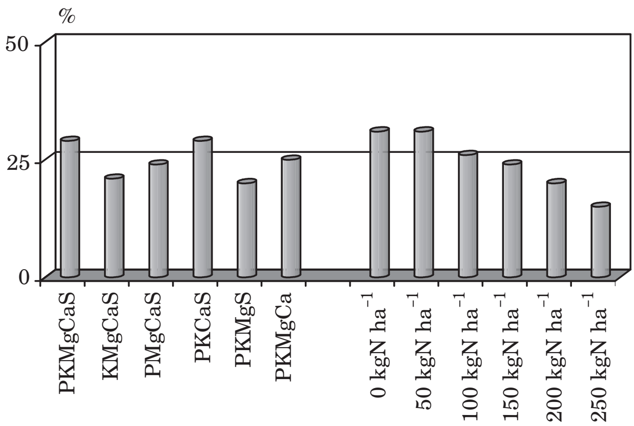
Table 2

The content of total phosphorus ($P_{tot}$), organic phosphorus ($P_{org}$) and available phosphorus ($P_{E-R}$) of investigated lessive soil depending on differentiated mineral fertilization (P, K, Mg, Ca, S) and increasing nitrogen doses

| Specification | | Phosphorus | | |
|---|---|---|---|---|
| | | total ($g\ kg^{-1}$) | organic ($g\ kg^{-1}$) | available ($mg\ kg^{-1}$) |
| Mineral fertilization I factor | P K Mg Ca S | 0.389 | 0.111 | 73.33 |
| | K Mg Ca S | 0.271 | 0.057 | 44.21 |
| | P Mg Ca S | 0.350 | 0.085 | 50.15 |
| | P K Ca S | 0.330 | 0.097 | 57.50 |
| | P K Mg S | 0.304 | 0.062 | 49.10 |
| | P K Mg Ca | 0.316 | 0.079 | 60.87 |
| Nitrogen ($kg\ ha^{-1}$) II factor | 0 | 0.366 | 0.113 | 77.83 |
| | 50 | 0.346 | 0.107 | 72.71 |
| | 100 | 0.340 | 0.089 | 59.96 |
| | 150 | 0.320 | 0.078 | 50.37 |
| | 200 | 0.305 | 0.062 | 42.58 |
| | 250 | 0.284 | 0.042 | 32.01 |
| Mean | | 0.327 | 0.082 | 55.85 |
| $LSD_{0.05}$ | | | | |
| I factor | | 0.003 | 0.003 | 1.614 |
| II factor | | 0.003 | 0.003 | 1.614 |
| Interaction | | | | |
| I/II | | 0.008 | 0.008 | 3.955 |
| II/I | | 0.008 | 0.008 | 3.955 |

Mineral fertilization alone, accompanied by increasing nitrogen rates, decreased the content of phosphorus in organic compounds in soil (Table 2). MCDOWELL, MONAGHAN (2002) found that nitrogen application at the rates of 200 kg N $ha^{-1}$ and 400 kg N $ha^{-1}$ resulted in a phosphorus decrease by 12% and 6%, respectively, compared with the control.

In most arable soils, phosphorus contained in organic bonds accounts for 20-50% of total P, but in the present research the share of organic phosphorus compounds in the total content of this nutrient was 15%-31%, depending on the fertilization (Figure 1). This result was attributed to the application of exclusive mineral fertilization, since in both natural and organic fertilizers nutrients occur mostly in organic compounds and are taken up by plants owing to microbiological and biochemical mineralization.




Rys. 1 Percentage of phosphorus in organic compounds in the total phosphorus concentration in the analyzed soil

The content of available phosphorus was 55.85 mg $P_{E\text{-}R}$ $kg^{-1}$, which classified the soil as class III, with an average content of this nutrient, according to PN-R-04023 (1996) (Table 2). On the other hand, a decrease in the $P_{E\text{-}R}$ content (44.21 mg $P_{E\text{-}R}$ $kg^{-1}$) was observed in the soil without phosphorus fertilization (K Mg Ca S variant), depressing the soil abundance from average to low. In the soil where maize was grown without Ca fertilization (P K Mg S variant), the $P_{E\text{-}R}$ content was 33% lower than in the variant with complete fertilization (P K Mg Ca S). The lack of liming, causing a lower soil pH, depressed the activation of soil phosphorus reserves by stimulating the transition of available forms into those hardly available to plants.

The variant without sulphur fertilization had a higher content of available phosphorus (60.87 mg $P_{E\text{-}R}$ $kg^{-1}$) than the other fertilization combinations except for complete mineral nutrition. As reported by FILIPEK and SKOWROŃSKA (2009), sulphur oxidation in soil increases the concentration of $H^{+}$ ions, which can lower the soil pH to 2-3, thus decreasing the P content in soil (SKWIERAWSKA, ZAWARTKA 2009, MAJCHERCZAK et al. 2013). Similarly, according to KACZOR, ŁASZCZ-ZAKORCZMENNA (2009), sulphur deficit in soil can limit the uptake of basic nutrients, including phosphorus, by plants. A lower supply of plants with sulphur limited the phosphorus uptake by plants, so that the soil from the sulphur unfertilized treatments was very rich in available phosphorus form.

Soil from the treatments with nitrogen fertilization was found to contain far less phosphorus available to plants, less by 33% on average for all the treatments, compared to the treatments not fertilized with this nutrient (Table 2), a result connected with the yield-stimulating effect of nitrogen and a resultant increase in the phosphorus uptake, accompanied by an increase in soil acidification caused by nitrogen fertilizers. In response to the increasing nitrogen rates, the soil changed its richness class from average to low. The lack of organic fertilization and unbalanced mineral fertilization decreased the content of the plant available phosphorus forms. Adding organic matter to soil alleviates the effects of unbalanced mineral fertilization and intensifies the processes of phosphate uptake by plants since FYM carbon releases phosphorus from organic bonds.

The statistical analysis confirmed a significant effect of the experimental factors on the changes in the activity of alkaline and acid phosphatase, the enzymes responsible for transformations of phosphorus in soil. In the soil taken up from the treatments where maize was fertilized without phosphorus (K Mg Ca S variant), the activity of both phosphatases was high (alkaline phosphatase 1.064 mM pNP $kg^{-1}$ $h^{-1}$, acid phosphatase 2.293 mM pNP $kg^{-1}$ $h^{-1}$).

According to ŻEBROWSKA et al. (2008), phosphorus deficit increases the production and secretion of extracellular acid phosphatases by plant roots to the substrate. No liming (P K Mg S variants) depressed the activity of alkaline phosphatase (0.844 mM pNP $kg^{-1}$ $h^{-1}$). At the same time, a decrease in soil pH occurred in non-limed treatments (Table 1). The low activity of alkaline phosphomonoesterase could be due to the sensitivity of phosphatases to soil reaction changes (WITTMANN et al. 2004). Increasing nitrogen rates significantly affected the activity of soil phosphatases, for example an inhibition of the activity of alkaline phosphatase was observed. A decrease in the enzymatic soil activity as a result of its acidification is due to the destruction of ion and hydrogen bonds in the active centre of an enzymatic protein. In turn, the activity of acid phosphatase was increasing to the nitrogen rate of 250 kg N $ha^{-1}$, when it reached the highest value (2.169 mM pNP $kg^{-1}$ $h^{-1}$) – Table 3. The enzymatic index of the soil pH was derived from the achieved values of the activity of acid and alkaline phosphatases (DICK et al. 2000). The value of the AlP:AcP ratio during the research was 0.312-0.741 (Table 3). The soil pH value considered optimal for the growth and development of plants ensures an adequate ratio of the AlP:AcP activity, namely 0.500. The value of the AlP:AcP ratio lower than 0.50 implies acid soil reaction and liming is recommended (DICK et al. 2000). Based on the enzymatic index of the soil pH calculated, it was noticed that the nitrogen rates above 100 kg N $ha^{-1}$ as well as the lack of Ca fertilization (P K Mg S variant) (AlP:AcP <0.500) acidified the soil. The soil pH index can be used as an alternative method to determine soil pH and its changes (LEMANOWICZ, KOPER 2009, LEMANOWICZ 2011).

The activity of acid phosphatase was closely correlated with the content of total phosphorus and its forms. The value of the correlation coefficient between the activity of AcP and the $P_{E-R}$ content in soil was $r$=-0.83, $p$<0.05, which corroborates earlier reports by FUKUDA et al. (2001) and KIELISZEWSKA-ROKICKA (2001), showing that the activity of soil phosphatases is usually reversely proportional to the content of mineral phosphorus. In response to

Table 3

The activity of alkaline (AlP) and acid (AcP) phosphatases and the AlP:AcP ratio in the investigated lessive soil depending on differentiated mineral fertilization (P, K, Mg, Ca, S) and increasing nitrogen doses

| Specification | | Phosphatases (mM pNP $kg^{-1}$ $h^{-1}$) | | AlP:AcP |
|---|---|---|---|---|
| | | Alkaline | Acid | |
| Mineral fertilization I factor | P K Mg Ca S | 0.909 | 1.558 | 0.583 |
| | K Mg Ca S | 1.064 | 2.293 | 0.464 |
| | P Mg Ca S | 0.871 | 1.690 | 0.515 |
| | P K Ca S | 0.877 | 1.741 | 0.504 |
| | P K Mg S | 0.844 | 1.835 | 0.459 |
| | P K Mg Ca | 1.001 | 1.914 | 0.523 |
| Nitrogen (kg $ha^{-1}$) II factor | 0 | 1.167 | 1.575 | 0.741 |
| | 50 | 1.137 | 1.630 | 0.698 |
| | 100 | 0.986 | 1.778 | 0.555 |
| | 150 | 0.860 | 1.882 | 0.473 |
| | 200 | 0.742 | 1.998 | 0.371 |
| | 250 | 0.676 | 2.169 | 0.312 |
| Mean | | 0.928 | 1.838 | |
| $LSD_{0.05}$ | | | | |
| I factor | | 0.011 | 0.034 | |
| II factor | | 0.011 | 0.034 | |
| Interaction | | | | |
| I/II | | 0.026 | 0.083 | |
| II/I | | 0.026 | 0.083 | |

the deficit of phytoavailable phosphorus, plants synthesize acid phosphatases, which stimulate transformations of organic phosphorus compounds into inorganic ones, directly available to plants. However, plant species differ in the amount and activity of the enzyme secreted. A significantly high coefficient of the correlation between the soil content of phosphorus in organic compounds and the available phosphorus form in soil was determined ($r$=0.95; $p$<0.05). Based on the significant values of the correlation coefficients between the activity of acid phosphatase and the content of phosphorus, it was concluded that the activity of this enzyme was a good parameter for characterization of soil, unlike the activity of alkaline phosphatase. According to Yadav and Tarafdar (2001), the activity of phosphatases can be used as an index to determine the degree of mineralization of organic phosphorus compounds. At the same time, phosphatases could be used for diagnosing the content of inorganic phosphorus in soils.

## CONCLUSIONS

1. Significant effect of mineral and nitrogen fertilization on changes in the content of total, organic and available phosphorus was determined. The highest content of phosphorus and its forms was shown in the soil with complete mineral fertilization composed of P K Mg Ca S and treated with 0 and 50 kg N $ha^{-1}$.

2. Unbalanced nitrogen fertilization without phosphorus nutrition decreased the content of the available form of phosphorus, changing the class of P richness of soil from average to low.

3. Determination of the activity of acid and alkaline phosphatases could aid long-term monitoring of the phosphorus status of soil and changes in soil abundance due to anthropogenic factors.

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