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Phosphatases activity and plant available phosphorus in soil under winter wheat (*Triticum aestivum* L.) fertilized minerally

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Abstract. The aim of this study was to determine the effect of mineral fertilization on the activity of alkaline and acid phosphatase and content of available phosphorus in soil under winter wheat cultivation. The first factor of experiment was the fertilization with P, K, Mg, Ca and S in 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). Second factor was the nitrogen fertilization in the form of ammonium nitrate (34% N) at doses: 0, 40, 80, 120, 160, 200 kg N ha^{-1}. Soil samples were assayed for the activity of alkaline and acid phosphatase and for the content of available phosphorus by Egner-Riehm method (DL). The lowest content of available phosphorus was found in soil without P fertilization (K, Mg, Ca, and S). In the soil from those treatments, the activity of phosphomonoesterases was the biggest. A low content of available phosphorus in the soil was also obtained in treatments without Mg and Ca application. A significant effect of nitrogen fertilization on the activity of alkaline and acid phosphatase was found. Increasing doses of nitrogen resulted in the increased acid phosphatase activity while contributing to the reduction of alkaline phosphatase activity.

key words: soil, mineral fertilization, available phosphorus, alkaline and acid phosphatases, nitrogen

INTRODUCTION

The ruling by the Minister of Environment (Regulation..., 2002) introduced to the national legislation to bring it in line with the Nitrates Directive (91/676/ EEC), was the first step to reduce the negative impact of agriculture on the environment. Farming practice points to the preference for nitrogen as a primary yielding factor, but an excess of that nutrient causes water pollution by nitrates. In addition to nitrogen, phosphorus is an essential element that causes disturbances in the environment by contributing to eutrophication of water. Therefore, in order to protect surface and groundwater the EU is currently preparing a Phosphates Directive.

An excess of one mineral can cause or exacerbate a shortage of others, even at their optimal content in the soil. This phenomenon is called an ion antagonism. The imbalance between high doses of nitrogen, which is the main yielding element, and an insufficient level of fertilization with other nutrients can lead to soil degradation, and thus to lower yields. The aim of this study was to determine the effect of mineral fertilization with P, K, Mg, Ca and S, and increasing doses of nitrogen on the activity of alkaline and acid phosphatase and on the content of available phosphorus in soil under winter wheat cultivation.

MATERIAL AND METHODS

Samples of soil for the study were taken from a long-term experiment, established by the Department of Plant Nutrition and Fertilization of the Institute of Soil Science and Plant Cultivation in Puławy. The experiment is conducted in the Agricultural Experimental Station in Grabów upon Vistula. Soils on which RZD in Grabów is located are typical luvisols (PN-R-04033, 1998), classified as a very good rye complex. The experiment was conducted in a four-year rotation: winter wheat + intercrop, grain maize, spring barley, winter oilseed rape. The experiment was conducted as a two-factorial one, arranged in a randomized block design in two replications. The first factor was the fertilization with P, K, Mg, Ca and S in 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). Second factor was the nitrogen fertilization in the form of ammonium nitrate (34% N) at doses: 0, 40, 80, 120, 160, 200 kg $N \cdot ha^{-1}$.

Forms of fertilizers were the following: in the treatments with sulfur, phosphorus and potassium fertilizers were applied – single superphosphate and potassium sulphate, in the treatments without sulfur, phosphorus and potassium fertilizers were used, such as triple superphosphate

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and high percent potassium salt, in the treatments with Ca and Mg, a dolomite containing 30% CaO and 10% Mg was used, on the plots without Mg – calcium oxide at the amount of 200 kg CaO ha^{-1}, whereas in the cases of Ca absence a magnesium sulphate at 70 kg MgO ha^{-1} was used. The doses of minerals used in the experiment were the following: 80 kg P_2O_5 ha^{-1}, 140 K_2O ha^{-1}, 70 MgO ha^{-1}, and 200 CaO ha^{-1}. The dose of sulfur results from the amount of this component introduced with appropriate doses of P, K, and Mg. Soil samples from under the cultivation of winter wheat were collected twice: in June and October 2008.

In the soil material, the activity of alkaline phosphatase (AlP) and acid phosphatase (AcP) were determined by Tabatabai and Bremner method (1969), bioavailable phosphorus (P_{E-R}) by Egner-Riehm method (DL) (Lityński and Jurkowska, 1976), and pH in 1 M KCl by potentiometry.

The obtained results were subjected to analysis of variance, and the significance of differences between means was verified using Tukey's test at a confidence level of P = 0.05. For the calculation FR-ANALWAR program was used, based on Microsoft Excel.

RESULTS

Mineral fertilization, which was used in the experiment resulted in a slight change in soil exchangeable acidity. PH_{KCl} values ranged between 4.9 and 6.0, depending on fertilization (Table 1). On the basis of the value of pH_{KCl}, the tested soil can be classified as an acidic and slightly acidic.

The content of available phosphorus in the soil in June was an average of 41.3 mg P_{E-R} kg^{-1}, whereas in the soil collected in October it was higher by 33% amounting to 62.3 mg of P_{E-R} kg^{-1} (Fig. 1). Lower content of available phosphorus in the soil in June can be explained by the increased absorbing of this component by winter wheat. In October, when the test crop had already been harvested, there were crop residues remaining in the soil, enriching the soil in this component. According to the criteria contained in the PN-R-04023 (1996), the studied soil belongs to the class III with a medium-high content of P_{E-R}.

The highest content of available phosphorus in the soils from both periods was found in sites with full mineral fertilization (P, K, Mg, Ca, S): 53.1 mg of P_{E-R} kg^{-1} in June and 75.7 mg P_{E-R} kg^{-1} in October (Fig. 1).

In the soil collected from plots without potassium fertilization (P, Mg, Ca, S), the content of available phosphorus was significantly higher (44.7 mg P_{E-R} kg^{-1} in the soil in June, 62.7 mg P_{E-R} kg^{-1} in October) than in soils not fertilized with Mg or Ca. A significant reduction in the P_{E-R} in samples of soil without Ca and Mg fertilization compared to full fertilized treatments was determined. In strongly acidic soil (fertilized with nitrogen at 200 kg N ha^{-1}) the use of liming and fertilization with Mg had a positive effect on the content of available phosphorus (Fig. 1).

A significant decrease was found in the content of available phosphorus in the treatments without sulfur fertilization (treatments P, K, Mg, Ca), collected in June (41.8 mg P_{E-R} kg^{-1}), and in October (72.4 mg P_{E-R} kg^{-1}), in comparison with full mineral fertilized treatments (P, K, Mg, Ca, S) (Fig. 1).

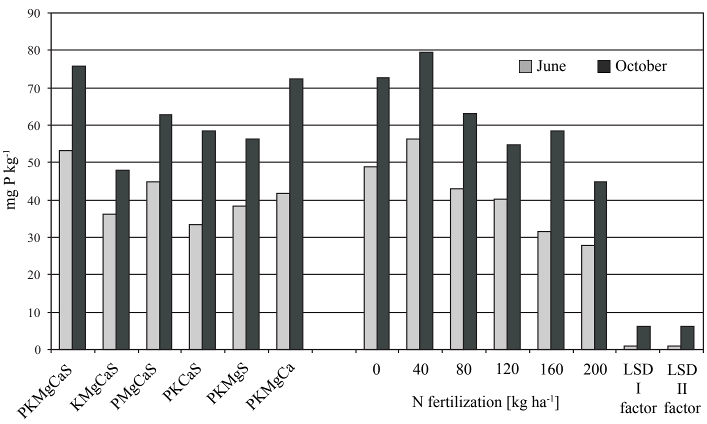

Fig. 1. The content of available phosphorus in the soil depending on fertilization.

Table 1. Exchangeable acidity of investigated lessive soil as depended on differentiated mineral fertilization (P, K, Mg, Ca, and S) and increasing nitrogen doses.

Nitrogen [kg 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	5.9	5.7	5.6	6.0
40	5.8	5.9	5.9	5.7	5.5	5.9
80	5.7	5.8	5.8	5.6	5.5	5.9
120	5.8	5.8	5.8	5.5	5.3	5.9
160	5.4	5.9	5.8	5.5	5.2	5.8
200	5.2	5.7	5.7	5.4	4.9	5.7

Table 2. The activity of alkaline and acid phosphatases and the ratio of alkaline to acid phosphatase AlP: ACP of investigated lessive soil as affected by differentiated mineral fertilization (P, K, Mg, Ca, and S) and increasing nitrogen doses.

Description		Alkaline phosphatase [mM pNP kg^{-1} h^{-1}]		Acid phosphatase [mM pNP kg^{-1} h^{-1}]		AlP:AcP	
		June	October	June	October	June	October
Mineral fertilization I factor	P K Mg Ca S	0.811	0.790	1.312	1.512	0.62	0.52
	K Mg Ca S	0.936	0.933	1.742	1.874	0.54	0.50
	P Mg Ca S	0.874	0.856	1.489	1.494	0.59	0.57
	P K Ca S	0.803	0.891	1.478	1.821	0.54	0.49
	P K Mg S	0.784	0.869	1.262	1.845	0.62	0.47
	P K Mg Ca	0.695	0.786	1.229	1.337	0.57	0.59
Nitrogen [kg ha^{-1}] II factor	0	0.927	0.962	1.102	1.377	0.84	0.70
	40	0.868	0.909	1.176	1.488	0.74	0.61
	80	0.832	0.881	1.364	1.583	0.61	0.56
	120	0.801	0.843	1.482	1.662	0.54	0.51
	160	0.759	0.793	1.597	1.780	0.48	0.45
	200	0.715	0.739	1.790	2.031	0.40	0.36
Mean		0.817	0.854	1.419	1.654	0.58	0.52
LSD (0.05)	I factor	0.016	0.014	0.148	0.025		
	II factor	0.016	0.014	0.148	0.025		
Interaction	I/II	0.039	0.034	0.363	0.061		
	II/I	0.039	0.034	0.363	0.061		

Acid phosphatase activity was significantly the greatest in the soil sampled in June (1.742 mM pNP kg^{-1} h^{-1}) and in October (1.874 mM pNP kg^{-1} h^{-1}) from the treatments not fertilized with P (K, Mg, Ca, S) (Table 2). In the soil without Ca fertilization, a lower alkaline phosphatase activity (0.784 mM pNP kg^{-1} h^{-1} in June) was found. The greatest activity of alkaline phosphatase was obtained in the soil not fertilized with nitrogen (0.927 mM pNP kg^{-1} h^{-1} in June and 0.962 mM pNP kg^{-1} h^{-1} in October).

The use of the highest dose of nitrogen (200 kg N ha^{-1}) resulted in a significant increase in acid phosphatase activity by 35% compared to soil not fertilized with N.

On the basis of alkaline phosphatase and acid phosphatase activity, a value of AlP:AcP ratio was calculated, known as an enzyme pH indicator (Dick et al., 2000). The value of AlP:AcP ratio was 0.36–0.84 during the study period. As optimum for plant growth and development, a soil pH can be regarded at which the correct ratio of AlP:AcP activities occurs (Dick et al., 2000). Ratio of AlP:AcP lower than 0.50 indicates an acidic soil where liming is recommended. The smallest ratio of the AlP:AcP was obtained in the soil taken from the treatments not fertilized with Ca (AlP:AcP = 0.47) and Mg (AlP:AcP = 0.49) as well as from those fertilized with the highest dose of nitrogen (200 kg N ha^{-1} – AlP:AcP = 0.36). An enzyme pH indicator can be used as an alternative method to determine pH of soil and changes occurring in it.

DISCUSSION

The research made it possible to assess the impact of fertilization on the mineral content of available phosphorus for plants and the activity of alkaline and acid phosphatase and in the soil under winter wheat.

According to Felczyński (2005) sulphate ions from the potassium fertilization cause precipitation of larger amounts of aluminium, and ultimately, the released Al binds phosphorus in aluminium phosphates unavailable for plants. Therefore, in soils where no fertilizer was applied, there was an increase of phosphorus available to plants. Potassium fertilization, through the influence on the increase of concentration of H^+ (the disclosure of exchangeable and physiological acidity) may indirectly affect changes in the bioavailability of nutrients (Skowrońska, Filipek, 2009).

Changes in the physicochemical and biological properties of soils occur under the influence of liming. Sorption capacity increases, as well as buffering and resistance to degradation, mineralization of organic matter is accelerated, thereby increasing absorption of phosphorus by plants (Skowrońska, Filipek, 2009). According to Bednarek and Reszka (2007), lack of liming increases the concentration of soluble and exchangeable Fe and Al, which may react with phosphorus, which leads consequently to the formation of poorly soluble phosphates of aluminium and iron in the soil. Filipek and Skowrońska (2009) state that oxidation of sulfur in the soil causes an increase in the concentration of H + ions, thereby reducing the supply of available phosphorus. Skwierawska and Zawartka (2009a) reported that in the 0–40 cm soil layer a sulfur dose of S-SO_4 and S-S^0 of 120 kg S ha^{-1} reduced the soil pH. Skwierawska and Zawartka (2009b) also said that only a dose of 120 kg ha^{-1} S-SO_4 affect the mobilization and migration of phosphorus in the soil. Sulfur deficiency in the soil can limit the uptake of basic nutrients, including phosphorus, by plants.

Smaller supply of sulfur reduces the uptake of phosphorus by plants, and as a result the soil not fertilized with sulfur shows a high supply of available forms of phosphorus.

The answer to the shortage of available phosphorus in soil is the phosphatase biosynthesis by both plant roots and micro-organisms. Therefore, the activity of those enzymes is largely related to the concentration of soil phosphorus (Heflik et al., 2007, Żebrowska et al., 2008). Typically, soil phosphatase activity is inversely proportional to the amount of mineral phosphorus in the soil, because low levels of inorganic phosphorus in the soil increased activity of phosphatases (Kieliszewska-Rokicka, 2001). A significant effect of nitrogen fertilization on the activity of both alkaline and acid phosphatase in the soil was found. Increasing the nitrogen dose resulted in decreased activity of AlP, while the activity of acid phosphatase increased. Higher acid phosphatase activity can be explained by the fact that phosphomonoesterases are the enzymes most sensitive to changes in soil pH. Optimum soil pH for alkaline phosphatase activity is 9.0–11.0, and 4.0–6.5 for the acid phosphatase (Dick, Tabatabai, 1984; Wittmann et al., 2004). Kucharski (1997) points out that increasing doses of nitrogen to 240 kg N ha^{-1} consistently heightened the activity of acid phosphatase in the soil while reducing the activity of AlP. According to Fukuda et al. (2001) plants usually secrete acid phosphatase in the soil when the supply of available phosphorus is low.

CONCLUSIONS

1. Nitrogen at a dose higher than 40 kg $N \cdot kg^{-1}$ caused a reduction in available phosphorus content in the tested soils, particularly in objects not fertilized with phosphorus. Lack of mineral fertilization with phosphorus and the use of high doses of nitrogen can lead to depletion of the soil in this nutrient.
2. Lack of phosphorus fertilizer increased the activity of both alkaline and acid phosphatase in the studied soil.
3. No liming and Mg fertilization resulted in a significant reduction in available phosphorus in the soil, as well as in alkaline phosphatase activity, while increasing the activity of acid phosphatase.
4. No sulfur fertilization affected the increase in the content of available phosphorus in the soil. The decrease in the activity of both studied enzymes was determined at these objects.

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