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SULPHUR AND PHOSPHORUS CONTENT AS WELL AS THE ACTIVITY OF HYDROLASES IN SOIL FERTILISED WITH MACROELEMENTS

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Abstract

Arylsulphatase and phosphatase may give some indication of the soil potential to perform specific biochemical reactions, and are also important contributors to soil fertility. Soil was collected from a field experiment set up at the Agricultural Experiment Station at Grabów by the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy. Soils samples from fields cropped with spring barley and winter wheat were collected in October 2008. The experiment involved only mineral fertilisation: increasing doses of ammonium nitrate (N0 – 0 kg N ha^{-1} to 150 kg N ha^{-1} for spring barley and N0 – 0 kg N ha^{-1} to 200 kg N ha^{-1} for winter wheat) and fertilisation with P – 30.5 kg P ha^{-1}, K – 116.2 kg K ha^{-1}, Ca – 143 kg Ca ha^{-1}, Mg – 42.2 kg Mg ha^{-1} and 30 kg S ha^{-1}. Sulphate sulphur was determined with the Bardsley-Lancaster method, while available phosphorus as well as the activity of arylsulphatase, acid phosphatase and alkaline phosphatase were assayed according to Tabatabai and Bremner. An effect of fertilisation with macronutrients and with increasing doses of nitrogen on the content of sulphates in soil was found in the fields cropped with either test plant. The highest content of sulphates was assayed in the soil from the treatments fertilised with macronutrients, except for Ca in soil under spring barley and Mg - under wheat. In the soil sampled under the test plants untreated with nitrogen, the content of available phosphorus was the highest. Increasing nitrogen doses significantly decreased the content of P_{E-R} and sulphates. The Polish Norm classifies it as soils of a medium content of phosphorus. It was demonstrated that the soil content of sulphates available to plants is affected by the presence of ions derived from fertilisation with Ca, Mg and K. This is most probably due to the sorption of sulphates and their unavailability in the soil solution.

Keywords: arylsulphatase, acid phosphatase, alkaline phosphatase.

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INTRODUCTION

Soil is a living, dynamic, non-renewable resource and soil conditions influence food production, environmental efficiency and global balance (GIANFREDA et al. 2005). The quality of soil depends partly on its natural composition and partly on changes resulting from its use and management by people. Mineral and organic fertilisation improves the productivity of soil. Special importance is attributed to nitrogen fertilisation as this is the nutrient producing the strongest impact. However, the uptake and then effective use of nitrogen by plants require an adequate content of the other macronutrients in soil. Nitrogen (N), phosphorus (P) and sulphur (S) are used for mineral fertilisation as they are responsible for the synthesis of proteins and a number of essential vitamins and cofactors (KERTESZ, MIRLEAU, 2004). The interaction of N with P can be claimed to constitute the single most important nutrient interaction of practical significance (AULAKH, MALHI, 2005). Over the recent years, both in Poland and in other European countries, a deficit of available sulphur forms has been recorded in plant production (SCHERER 2009). The availability of S and P to plants is controlled by numerous factors that affect the dynamics of macroelement fractions in soil. Changes in the soil's inorganic S and P pool play a major role in the above dynamics owing to its potential impact on the transformation of organic fractions in soil (KERTESZ, MIRLEAU, 2004, AULAKH, MALHI 2005, SCHMIDT 2012). The transformation of S and P organic compounds to inorganic sulphate and phosphate (mineralisation) and a reverse process (incorporation of sulphate into soil organic compounds or immobilisation) play an important role in the cycle of macroelements within soil and both are microbiologically mediated (SINGH et al. 2007, SCHERER 2009). Arylsulphatase and phosphatase are essential for the mineralisation of sulphur and phosphorous compounds. They may give some indication of the soil potential to perform specific biochemical reactions, and are important in contributing to soil fertility (GIANFREDA et al. 2005, SAVIOZZI et al. 2006).

The aim of this paper has been to determine the extent to which fertilisation with increasing doses of nitrogen as well as fixed doses of phosphorus, potassium, calcium, magnesium and sulphur under spring barley and winter wheat affects the soil richness with phytoavailable forms of sulphur and phosphorus as well as the activity of hydrolases participating in the transformations of those elements in soil.

MATERIAL AND METHODS

Site description and experimental design

Controlled field trials were performed on a field used for a long-term experiment and located at the Agricultural Experiment Station at Grabów, the Province of Mazowsze (*województwo mazowieckie*). The exact geographical location of the experimental station is 51°21'8"N latitude and 21°40'8"E longitude. The soils at the Experimental Station in Grabów are Haplic Luvisols (LVh) classified as light loamy and sandy in texture according to the USDA soil classification (18% silt and clay fraction and 1.39% organic matter content). The experiment was performed in a four-year rotation cycle: grain maize, spring barley, winter oilseed rape, winter wheat + intercrop. A two-factors experiment in a randomised block design was carried out in two replications. The first factor involved P, K, Mg, Ca and S fertilisation 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 fertilisation at these levels: spring barley: N0 – 0, N1 – 30, N2 – 60, N3 – 90, N4 – 120, N5 – 150 kg N ha^{-1}; winter wheat: N0 – 0, N1 – 40, N2 – 80, N3 – 120, N4 – 160, N5 – 200 kg N ha^{-1}. The following fertiliser forms were applied: phosphorus and potassium fertilisers containing sulphur such as single superphosphate and potassium sulphate in treatments with sulphur; phosphorus and potassium fertilisers which did not include sulphur such as tringle superphosphate, and high-percentage potassium salt in treatments without sulphur; dolomite containing 21.3% Ca and 6% MgO in treatments with Ca and Mg; lime was used in the amount of 143 kg Ca ha^{-1} on plots without Mg, and magnesium sulphate was supplied at 42 kg Mg ha^{-1} on plots without Ca. The doses of minerals applied in the experiment were as follows: with P – 30.5 kg P ha^{-1}, K – 116.2 kg K ha^{-1}, Ca – 143 kg Ca ha^{-1}, Mg – 42.2 kg Mg ha^{-1} and 20 kg S ha^{-1}. Soils samples from the fields cropped with spring barley and winter wheat were collected in October 2008.

Analysis of soil properties

The following were assayed in prepared soil material: sulphate sulphur according to the Bardsley-Lancaster method (Bardsley, Lancaster 1960) modified by COMN-IUNG. Sulphate sulphur (SO_4^{2-}) was determined by the turbidimetric method. Soil samples were extracted with CH_3COONH_4 dissolved in CH_3COOH by shaking for 30 minutes. The filtrate solution mixed with $BaCl_2$ was stirred, left aside for 15 min, after which the turbidity was measured at 440 nm using a spectrophotometer. The content of available phosphorus (P_{E-R}) (PN-R-04023, 1996) was measured spectrophotometrically, by testing the intensity of the colour of phosphorus-molybdenum blue created by orthophosphoric ions with molybdenum ions in an acid environment in the presence of $SnCl_2$. The activity of arylsulphatase (ARS) was determined according to the Tabatabai and Bremner method (Tabatabai, Bremner 1970), while the activity of acid (AcP) and alkaline (AlP) phosphatase was assessed with the protocol described earlier by the same authors (Tabatabai, Bremner 1969). In both cases, the procedure was based on colorimetric estimation of *p*-nitrophenol released by enzymatic activity when soil was incubated at 37°C with a buffer and substrate specific for each enzyme (potassium-*p*-nitrophenylsulphate salt for arylsulphatase and p-nitrofenylophosphate for the phosphatases). After 1 h of incubation, the reaction was stopped and the intensity of the yellow colour of the filtrate was measured spectrophotometrically. Finaly, pH 1 mol dm^{-3} KCl was determined potentiometrically (PN-ISO 10390 1997).

Statistical analysis

The data underwent analysis of variance and the significance of differences between the means was defined with the Tukey's test at the significance level $p = 0.05$. The calculations involved the use of Anova based on Microsoft Excel. Finally, the results were submitted to the analysis of simple correlation ($p < 0.05$), which determined the degree of dependence between respective factors. Correlation analysis was performed using Statistica for Windows Pl software.

RESULTS AND DISCUSSION

The reaction of soil collected from the experimental field in Grabów ranged from 5.9 to 6.9 under spring barley and 4.9 to 6.0 under winter wheat, depending on fertilisation (Table 1). Thus, it ranged from being acid and slightly acid to neutral. No changes in the soil reaction were noticed due to the increasing doses of ammonium nitrate or the various combinations of P, K, Ca, Mg and S fertilisation tested in this experiment. Fertilisers containing

Table 1

Reaction (pH in 1mol KCl dm^{-3}) of soil in the years of the investigation

Treatments I factor	The level of nitrogen fertilization											
	spring barley						winter wheat					
	N0	N1	N2	N3	N4	N5	N0	N1	N2	N3	N4	N5
1.	6.3	6.5	6.4	6.8	6.6	5.9	5.8	5.8	5.7	5.8	5.4	5.2
2.	6.5	6.3	6.4	6.9	6.3	5.8	5.9	5.9	5.8	5.8	5.9	5.7
3.	6.2	6.5	6.7	6.2	6.4	5.9	5.9	5.9	5.8	5.8	5.8	5.7
4.	6.7	6.5	6.6	6.6	6.6	5.9	5.7	5.7	5.6	5.5	5.5	5.4
5.	6.7	6.3	6.6	6.4	6.6	5.6	5.6	5.5	5.5	5.3	5.2	4.9
6.	6.8	6.5	6.7	6.4	6.6	6.1	6.0	5.9	5.9	5.9	5.8	5.7

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 -

sulphur can decrease soil pH. Adsorption of sulphate is pH-dependent, being more intensive at low soil pH (Scherer 2009).

The content of sulphur available to plants in the soil cropped with spring barley was 10.32 mg kg^{-1} on average (Table 2), which classifies it as soils of a medium content of sulphur. Soil under winter wheat contained 8.611 mg S kg^{-1} on average (mean values for all the treatments), which classifies it as soil of a low content of sulphur. In line with the fertilisation guidelines, to ensure high and good quality cereal yield, these soils should be enriched with sulphur at the amount of 25 kg S ha^{-1} for spring barley as well as 30 kg S ha^{-1} for wheat (Lipinski et al. 2003).

Fertilisation with macronutrients and with nitrogen affected the content of sulphates in soil (Table 2). The highest content of sulphates was detected in the soil from the treatments without Ca (treatment 5) cropped with spring barley (14.51 mg kg^{-1} SO_4^{2-} on average) and without Mg (treatment 4) cropped with wheat (11.26 mg kg^{-1} SO_4^{2-} on average). Simultaneous application of Ca and Mg with S may contribute to a more permanent binding of sulphates in the soil sorption complex. According Kovar and Grant (2011), sulphates can be present in the soil solution, adsorbed on the surface of minerals or coprecipitated with Ca and Mg. With respect to both cereals, the lowest content of sulphates appeared in the soil taken from the plots without K fertilisation (treatment 3). The content of available sulphur from these treatments was 34% under barley and 22% under wheat, thus being lower than in the fertilisation regime that excluded sulphur (treatment 6). According to Taqi et al (2011), sulphur is a vital ingredient in nutrient management plans designed for high-yield, top profit cropping systems and an adequate amount of this element is especially necessary for attaining an optimum plant use of N and K. Most probably, the content of K ions in soil also affects the sorption and leaching of sulphate ions in soil. An earlier report by Eriksen (2009) shows that sulphate adsorption is influenced by the presence of other anions: phosphate > nitrate = chloride. The increasing HPO_4^{2-} concentration in the soil solution containing both SO_4^{2-} and HPO_2^{4-} decreased SO_4^{2-} adsorption and because adsorbed sulphates are attached far less strongly than adsorbed HPO_2^{4-}, application of P fertilisers, like liming, increases the availability of SO_4^{2-} (Scherer 2009). Research performed in Luvisols demonstrated such a considerable effect of fertilisation with P. The content of sulphates in the trials without that macronutrient (treatment 2), under both cereals, showed the same content of sulphates as in the case of the variant with full fertilisation including all the macronutrients (treatment 1). However, an effect of N fertilisation on the content of sulphates was noticed (Table 2). N and S are closely interrelated in the plant - soil system (protein synthesis and soil organic matter constituents), so the availability of both nutrients is expected to affect the dynamics of the soil S pools (Schmidt 2012). In our experiment, the increasing nitrogen doses decreased the content of sulphur available to plants. The highest content of this form of the element was noted in the soil fertilised with N1 for barley and N0 for wheat. In the soil sampled from tre-

Table 2

Content of sulphate sulphur and activity of arylsulphatase

Treatments I factor	Spring barley							Winter wheat						
	the level of nitrogen fertilization II factor													
	N0	N1	N2	N3	N4	N5	mean	N0	N1	N2	N3	N4	N5	mean
	Sulphate sulphur content ($mg \cdot kg^{-1}$)													
1.	15.65	6.808	10.93	13.39	10.14	9.399	11.05	6.749	7.638	8.228	6.636	6.819	8.226	7.383
2.	8.435	6.808	7.820	11.91	9.353	11.56	11.05	8.621	7.831	7.494	6.898	6.060	7.156	7.343
3.	9.670	13.27	9.410	5.045	7.189	4.734	8.219	7.775	7.381	5.071	9.184	5.466	7.494	7.062
4.	9.363	5.410	11.83	7.393	12.60	14.51	10.22	12.32	12.60	11.74	11.41	8.678	10.80	11.26
5.	10.01	15.88	9.680	14.04	13.62	11.67	12.50	10.59	8.315	10.55	11.11	8.970	7.550	9.513
6.	12.34	13.65	9.764	12.31	8.611	6.875	10.59	10.26	8.903	8.825	9.490	7.888	9.296	9.109
x	10.92	13.00	9.906	10.68	10.25	9.832	10.32	9.385	8.777	8.650	9.120	7.313	8.420	8.611
	$LSD_{0.05}$ I - 0.258 II - 0.258 I/II - 0.632 $LSD_{0.05}$ I - 0.333 II - 0.333 I/II - 0.815													
	Arylsulphatase activity (mM pNP kg^{-1} h^{-1})													
1.	0.052	0.044	0.027	0.033	0.066	0.047	0.045	0.071	0.072	0.062	0.068	0.059	0.059	0.065
2.	0.076	0.071	0.057	0.050	0.056	0.065	0.063	0.072	0.068	0.071	0.047	0.067	0.064	0.065
3.	0.041	0.045	0.034	0.058	0.045	0.040	0.044	0.081	0.063	0.063	0.063	0.059	0.075	0.068
4.	0.072	0.033	0.050	0.049	0.036	0.052	0.049	0.082	0.051	0.062	0.068	0.066	0.040	0.061
5.	0.066	0.040	0.043	0.057	0.062	0.062	0.055	0.064	0.068	0.072	0.061	0.071	0.058	0.066
6.	0.063	0.059	0.065	0.068	0.062	0.054	0.062	0.071	0.070	0.062	0.062	0.066	0.075	0.071
x	0.062	0.049	0.046	0.053	0.055	0.054	0.053	0.074	0.065	0.065	0.061	0.062	0.062	0.065
	$LSD_{0.05}$ I - 0.005 II - 0.005 I/II - 0.013 $LSD_{0.05}$ I - n.s. II - 0.006 I/II - 0.015													

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 -

atment N5, the content of sulphur was 24% lower in the barley field and 10% lower in the wheat field. Likewise, Jamal et al (2006) showed that large doses of nitrogen created sulphur deficiency.

The average content of available phosphorus in the soil was 58.73 mg kg^{-1} P (mean values for spring barley) and 55.17 mg P kg^{-1} (mean values for winter wheat), which, according to PN-R-04023 (1996), classifies it as soil of a medium phosphorus content (class III) – Table 3. A significant effect of the fertilisation on changes in the content of P_{E-R} was found. The highest accumulation of this macronutrient was recorded in the soil under wheat (67.18 mg kg^{-1} P on average) and barley (75.51 mg kg^{-1} P on average) with full mineral fertilisation (treatment 1). According to Singh et al. (2007), long-term application of phosphorus fertiliser in excess of a crop's requirement can build up large amounts of P in soil. Mineral fertilisation without phosphorus (treatment 2) significantly decreased the content of P_{E-R} in soil under spring barley (by 41%; 44.53 mg kg^{-1} P on average) and under wheat (by 36%; 42.77 mg kg^{-1} P on average) – Table 3, which lowered the soil richness class from III (medium) to IV (low). Similarly, mineral fertilisation without Ca (treatment 5) supplied to barley and wheat decreased the content of the available phosphorus form. The application of fertilisation without sulphur (treatment 6) increased P_{E-R} in soil under both cereal plants (61.18 mg kg^{-1} P for spring barley as well as 55.87 mg kg^{-1} P for wheat). According to Kozłowska-Strawska (2007), an excessive amount of sulphur introduced into soil decreases the pH value in soil, which can lead to changes in the content of available forms of nutrients. Also Majcherczak et al. (2013) demonstrated that sulphur doses considerably decreased the content of available forms of phosphorus, potassium and magnesium in soil.

The content of available phosphorus was the highest in the soil sampled under the test plants untreated with nitrogen. The increasing nitrogen doses significantly decreased the content of P_{E-R}. The highest dose of nitrogen N5 decreased the content of P_{E-R} by an average of about 48%, as compared with the level of nitrogen fertilisation N0. The effect of nitrogen, on the one hand, can be viewed in terms of its impact on growing yields and a higher phosphorus uptake. On the other hand, high N doses which can increase soil acidity, and the capacity for phosphorus adsorption by hydrated aluminium and iron oxides is higher at a low soil reaction (Emnova et al. 2014). In turn, a higher concentration of phosphate anions, being the consequence of a less intensive binding of phosphorus, occurs in soils with pH ranging from 6 to 7. According to Liu et al. (2010), supplying only N fertilisers may result in a deficiency of other nutrients and a decline in soil's chemical, biological and physical properties. Previous studies confirmed that fertilisation with increasing nitrogen doses raised the activity of acid phosphatase and inhibited the activity of alkaline phosphatase in surface soils (Lemanowicz et al. 2014).

Fertiliser macroelements significantly affected arylsulphatase, soil alkaline and acid phosphatase activities (Table 3). The activity of arylsulphatase was (on average for all the treatments) 14% higher in the soil sampled under

Table 3

Content of available phosphorus and activity of alkaline and acid phosphatase

Treat-ments I factor	Spring barley							Winter wheat						
	the level of nitrogen fertilization II factor													
	N0	N1	N2	N3	N4	N5	mean	N0	N1	N2	N3	N4	N5	mean
Available phosphorus content (mg kg^{-1})														
1.	99.46	95.10	89.66	60.26	59.14	49.46	75.51	93.75	80.86	77.94	48.15	47.27	55.09	67.18
2.	60.77	53.78	49.59	47.38	41.68	13.99	44.53	54.70	49.88	40.21	48.38	37.02	26.42	42.77
3.	70.29	67.02	64.25	61.98	51.49	48.43	60.58	67.82	56.71	54.09	52.30	49.30	43.77	53.50
4.	72.37	70.06	63.88	66.35	68.20	44.42	63.71	77.07	73.61	70.30	59.49	52.08	31.79	60.72
5.	78.82	64.93	49.88	37.10	27.67	22.73	46.85	63.50	59.46	56.20	53.44	48.05	32.91	52.26
6.	58.34	64.73	51.54	78.16	68.87	45.87	61.18	76.00	70.70	62.07	58.96	34.48	33.01	55.87
x	73.34	69.27	61.47	58.54	52.27	37.49	58.73	71.64	65.20	60.13	53.45	44.70	37.17	55.38
$LSD_{0.05}$ I - 1.602 II - 1.602 I/II - 3.924 $LSD_{0.05}$ I - 1.119 II - 1.129 I/II3.011														
Alkaline phosphatase activity (mM pNP kg^{-1} h^{-1})														
1.	0.820	0.932	0.809	0.773	0.747	0.632	0.785	0.815	0.754	0.688	0.647	0.616	0.583	0.684
2.	1.028	0.937	0.873	0.830	0.820	0.792	0.880	1.160	0.985	0.877	0.791	0.687	0.624	0.854
3.	0.731	0.720	0.700	0.673	0.651	0.547	0.670	0.759	0.697	0.647	0.580	0.516	0.485	0.614
4.	0.822	0.797	0.768	0.726	0.711	0.541	0.727	0.653	0.624	0.587	0.523	0.486	0.430	0.550
5.	0.744	0.828	0.683	0.726	0.612	0.494	0.681	0.575	0.579	0.530	0.467	0.425	0.370	0.491
6.	0.947	1.003	0.984	0.955	0.873	0.622	0.904	0.370	0.713	0.667	0.619	0.579	0.516	0.577
x	0.849	0.869	0.803	0.780	0.736	0.611	0.775	0.722	0.725	0.666	0.604	0.551	0.501	0.628
$LSD_{0.05}$ I - 0.154 II 0.154 I/II 0.317 $LSD_{0.05}$ I - 0.043 II- 0.043 I/II-0.106														
Acid phosphatase activity (mM pNP kg^{-1} h^{-1})														
1.	1.385	1.514	1.579	1.485	1.578	1.872	1.569	1.590	1.660	1.851	1.981	2.115	2.528	1.954
2.	2.005	2.462	2.596	2.646	2.104	2.850	2.444	1.028	0.937	0.873	0.830	0.820	0.792	0.880
3.	1.736	1.704	1.794	1.946	2.101	2.239	1.920	1.431	1.479	1.686	1.435	1.669	1.908	1.601
4.	1.649	1.825	1.995	2.086	2.184	2.256	1.999	1.108	1.209	1.310	1.374	1.470	1.533	1.334
5.	1.492	1.776	1.891	1.862	1.974	2.228	1.871	1.907	1.580	1.542	1.716	1.948	2.271	1.827
6.	1.145	1.569	1.607	1.720	1.594	1.833	1.578	1.431	1.478	1.986	1.435	1.669	1.907	1.601
x	1.569	1.808	1.910	1.957	1.922	2.213	1.897	1.416	1.390	1.491	1.462	1.615	1.823	1.533
$LSD_{0.05}$ I - 0.020 II - 0.020 I/II - 0.049 $LSD_{0.05}$ I - 0.018 II - 0.018 I/II - 0.044														

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 -

wheat than under spring barley. It can also result from the fact that in soil under wheat contained nearly 16% less sulphates (Table 2). According to Saviozzi et al. (2006), low SO_4^{2-} levels stimulate soil microorganisms to produce sulphatases or activate enzymes, a hypothesis supported by the results of the activity of arylsulphatase depending on fertilisation with various macroelements, since the highest activity of arylsulphatase was found in the soil cropped with wheat in the trial without S fertilisation (treatment 6), and in soil cropped with spring barley which received complete fertilisation with all the macronutrients (treatment 1) or without S fertilisation (treatment 6) – Table 2.

Both in barley and in wheat fields, the highest activity of arylsulphatase was found in the soil not fertilised with nitrogen, where it was 13% and 16% higher, respectively, than in treatments with the highest dose of nitrogen fertiliser.

A significant effect of nitrogen fertilisation on the activity of alkaline and acid phosphatase was observed (Table 3). The activity of acid phosphatase was higher in the soil under both spring barley, where it ranged 1.145-2.850 mM pNP kg^{-1} h^{-1} (on average for the experiment 1.897 mM pNP kg^{-1} h^{-1}) and wheat, where it was from 0.792 to 2.528 mM pNP kg^{-1} h^{-1} (on average for the experiment 1.533 mM pNP kg^{-1} h^{-1}) – Table 3. Similarly Lemanowicz and Bartkowiak (2013) point to changes in the enzymatic activity depending on the species composition of the plant cover and sometimes even on cultivars, which is connected with diverse species composition of soil microorganisms which inhabit the roots of plants as well as root secretions, thus affecting the concentration of soluble carbon in soil.

Balanced fertilisation with macroelements showed a significant effect on the changes in the activity of phosphatases (Table 3). The highest activity of alkaline phosphatase was identified in the soil under spring barley (0.880 mM pNP kg^{-1} h^{-1}) and winter wheat (0.864 mM pNP kg^{-1} h^{-1}) while the highest activity of acid phosphatase was determined under spring barley (2.444 mM pNP kg^{-1} h^{-1}) not fertilised with phosphorus (treatment 2). An increase in the secretion of phosphatases by soil microorganisms and plant roots exposed to phosphorus deficit was also observed by Ciereszko et al. (2011), which points to the secretion of those enzymes playing an essential role in increasing the availability and uptake of phosphorus from organic compounds in soil. A significant effect of nitrogen fertilisation on the changes in the activity of soil phosphatases was found. On the one hand, the increasing nitrogen doses inhibited AlP, but on the other hand, the activity of AcP was increasing. The decrease in enzymatic activities could be due to the acidifying effect of N fertilisers applied in the form of ammonium nitrate. Similar results were earlier presented by Liu et al. (2010). Soil enzymes were usually significantly correlated with soil pH. In our research, a moderate negative correlation between the activity of arylsulphatase and soil pH was observed (r = -0.452, p < 0.05) – Table 4. Elsewhere, positive, negative or no correlations have been reported (Gianfreda et al. 2005). Accordingly, the

Table 4

Correlation matrix between the parameters analysed ($n = 36$, $p < 0.05$)

Variables		Equation	r	r^2
Dependent	Independent			
Spring barley				
Alkaline phosphatase	available phosphorus	$y = 0.0029x+0.607$	0.404	0.162
Acid phosphatase	available phosphorus	$y = -0.0104x+2.487$	-0.558	0.311
pH	available phosphorus	$y = 0.0069x+6.007$	0.412	0.171
Arylsulphatase	alkaline phosphatase	$y = 0.0341x+0.026$	0.350	0.122
pH	alkaline phosphatase	$y = 1.134x+5.531$	0.483	0.234
pH	acid phosphatase	$y = -0.303x+6.978$	-0.339	0.115
Winter what				
Acid phosphatase	available phosphorus	$y = -0.0164x+2.622$	-0.561	0.315
pH	available phosphorus	$y =0.0076x+6.0016$	0.413	0.171
pH	arylsulphatase	$y = -16.45x+7.4486$	-0.456	0.208

arylsulphatase of agricultural soils was significantly and positively correlated with pH, whereas an opposite response (i.e. negative, significant correlation) was exhibited by phosphatase. Although phosphatase activity was determined at the natural soil pH, the endogenous soil phosphatase might show an optimal pH different from that used in the enzymatic assay. The analysis of correlations revealed a moderate positive significant dependence between the content of available phosphorus in soil and the activity of alkaline phosphatase ($r = 0.404$, $p < 0.05$) and a negative correlation between acid phosphatase in soil under spring barley ($r = -0.558$, $p < 0.05$) and under winter wheat ($r = -0.561$, $p < 0.05$), which suggests that those enzymes take part in soil phosphorus cycle.

The present research results are confirmed by many authors (Sienkiewicz et al. 2009, Majcherczak et al. 2013, Lemanowicz et al. 2014,), who conlcuded that farming practice should comply with the assumption of integrated agriculture, in which both mineral and organic or natural fertilisation regimes are implemented.

CONCLUSIONS

The highest content of sulphates and available phosphorus was accumulated in soil without fertilisation with ammonium nitrate (control) or with fertilisation using its lowest dose.

2. The treatment with P, Mg, Ca and S but without K (as sulphate potassium) resulted in the lowest content of sulphates in soil. The use of fertilisers containing sulphates and Ca or Mg contributed to an increasing content of sulphates in soil. Interactions between the nutrients could have

practical consequences, suggesting that K and Ca or Mg should be added to S fertilizers.

3. A high activity of arylsulphatase in the treatments without S fertilisation suggests that it is stimulated at a low content of sulphates in soil.

4. The increasing level of nitrogen fertilisation inhibited the activity of alkaline phosphatase in soil under winter wheat and increased the activity of acid phosphatase in soil under both plants. The activity of arylsulphatase depended on nitrogen fertilisation, and the highest activity of this hydrolase was found in soil without nitrogen fertilisation.

REFERENCES

Aulakh M.S., Malhi S.S. 2005. *Interactions of nitrogen with other nutrients and water: Effect on crop yield and quality, nutrient use efficiency, carbon sequestration, and environmental pollution*. Adv. Agron., 86: 341-409.

Bardsley C.E., Lancaster J.D. 1960. *Determination of reserve sulfur and solube sulfates in soil.* Soil Sci. Soc. Am. Proc., 24: 265-268.

Ciereszko I., Szczygła A., Żebrowska E. 2011. *Phosphate deficiency affects acid phosphatase activity and growth of two wheat varieties.* J. Plant Nutrit., 34(6): 815-829

Emnova E.E., Daraban O. V., Bizgan I.V., Toma S.I. 2014. *Effect of phosphoric fertilizer and starter rates of nitrogen fertilizers on the phosphatase activity in the rhizosphere soil and nonlignified soybean roots under drought conditions*. Eur. Soil Sci., 47(2):102-109. DOI: 10.1134/S1064229314020057

Eriksen J 2009. *Soil sulphur cycling in temperate agricultural system.* Adv. Agron. 102: 55-89

Gianfreda L,, Rao M. A., Piotrowska A., Palumbo G., Colombo C. 2005. *Soil enzyme activities as affected by anthropogenic alterations: intensive agricultural practices and organic pollution*. Sci. Total Environ., 341: 265-279.

Jamal A., Fazli I.S., Ahmad S., Abdin M.Z. 2006. *Interactive effect of nitrogen and sulphur on yield and quality of groungnut (Arachis hypogea* L.*).* Korean J. Crop Sci., 51(6): 519-522.

Kertesz M.A., Mirleau P. 2004. *The role of soil microbes in plant sulphur nutrition.* J. Exp. Bot., 55: 1939-1945.

Kozłowska-Strawska J. 2007. *Influence of plant fertilization with different sulphur compounds on the changes of soil reaction.* Zesz. Probl. Post. Nauk Rol., 520: 635-639. (in Polish)

Kovar J.L., Grant C.A. 2011. *Nutrient cycling in soils: Sulfur*. Publications from USDA-ARS/ UNL Faculty. Paper 1383. available: http://digitalcommons.unl.edu/usdaarsacpub/1383.

Lemanowicz J., Bartkowiak A. 2013. *Diagnosis of the content of selected heavy metals in the soils of the Pałuki region against their enzymatic activity.* Arch. Environ. Protect., 39(3): 23-32. DOI: 10.2478/aep-2013-0026

Lemanowicz J., Siwik-Ziomek A., Koper J. 2014. *Effects of farmyard manure and nitrogen fertilizers on mobility of phosphorus and sulphur in wheat and activity of selected hydrolases in soil.* Int. Agroph., 28: 49-55. DOI: 10.2478/intag-2013-0026

Lipiński W., Terelak H., Motowicka-Terelak T., 2003. *Suggestion for liming values of sulphate sulphur content in mineral soils for fertilization advisory needs.* Soil Sci. Ann., 54 (3): 79-84. (in Polish)

Liu E., Yan Ch., Mei X., He W., Bing S. H., Ding L., Liu Q., Liu S., Fan T. 2010. *Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China.* Geoderma, 158: 173-180. DOI:10.1016/j.geoderma.2010.04.029

Majcherczak E., Kozera W., Ralcewicz M., Knapowski W. 2013. *Content of total carbon and* *available forms of phosphorus, potassium and magnesium in soil depending on the sulphur rate and form*. J. Elem., 18(1): 107-114. DOI: 10.5601/jelem.2013.18.1.09

PN-R-04023. 1996. *Chemical and agricultural analysis of soil – determining the content of available phosphorus in mineral soils*. Polish Standards Committee, Warsaw. (in Polish)

PN-ISO 10390. 1997. *Chemical and agricultural analysis – determining soil pH*. Polish Standards Committee, Warsaw. (in Polish)

Saviozzi A., Cardeli R., Cipolli S., Levi-Minzi R., Riffaldi R. 2006. *Arylsulphatase activity during the S mineralization in soils amended with cattle manure and green waste*. Agrochimica, 50: 231-237.

Scherer H.W. 2009. *Sulfur in soils*. J. Plant Nutr. Soil Sci., 172: 326-335.

Schmidt F., De Bona F.D., Silveira C.P., Monteiro F.A. 2012. *Soil sulfur fractions dynamics and distribution in a tropical grass pasture amended with nitrogen and sulfur fertilizers*. J. Plant Nutr. Soil Sci., 175: 60-67.

Sienkiewicz S., Krzebietke S., Wojnowska T., Żarczyński P., Omilian M. 2009. *Effect of long-term differentiated fertilization with farmyard manure and mineral fertilizers on the content of available forms of P, K and Mg in soil*. J. Elem., 14(4): 779-786.

Singh M., Reddy S.R., Singh V.P., Rupa T.R. 2007. *Phosphorus availability to rice (Oriza sativa* L.*) and wheat (Triticum aestivum* L.*) in a Vertisol after eight years of inorganic and organic fertilizer additions*. Bioresour. Technol., 98: 1474-1481.

Tabatabai M.A., Bremner J.M. 1969. *Use of p–nitrophenol phosphate for assay of soil phosphatase activity*. Soil Biol. Bioch., 1: 301-307.

Tabatabai M.A., Bremner J.M. 1970. *Factors affecting soil arylsulfatase activity*. Soil Sci. Soc. Am. Proc., 34: 427-429.

Taqi A.K., Mohd M., Firoz M. 2011. *Sulphur management: An agronomic and transgenic approach*. J. Ind. Res. Tech., 1(2): 147-161.