
ARYLSULPHATASE ACTIVITY AND THE CONTENT OF TOTAL SULPHUR AND ITS FORMS UNDER THE INFLUENCE OF FERTILISATION WITH NITROGEN AND OTHER MACROELEMENTS

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Abstract

The aim of this research was to determine the effect of mineral fertilisation on the activity of arylsulphatase (EC 3.1.6.1.) and the content of total sulphur and its fraction in soil under winter wheat. The soil was collected from a field experiment set up at the Agricultural Experimental Station in Grabów nad Wisłą by the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy. The sampling was carried out twice during the vegetative period of maize in 2008. The experiment involved exclusive mineral fertilisation composed of increasing rates of ammonium nitrate (0, 50, 100, 150, 200 kg N ha⁻¹), and fertilisation with P – 80 kg P₂O₅ ha⁻¹, K – 140 kg K₂O ha⁻¹, Ca – 200 kg CaO ha⁻¹, Mg – 70 kg MgO ha⁻¹ and S in fertilisation forms with other elements. The activity of arylsulphatase was determined following the Tabatabai and Bremner method, and sulphate sulphur was assayed compliant with the Bardsley–Lancaster method. The fertilisation with ammonium nitrate determined the content of total sulphur and its fraction as well as the activity of arylsulphatase in Luvisol. The optimum nitrogen rate leading to the highest arylsulphatase activity in soil was the rate of 100 kg N ha⁻¹. The analysed Luvisol demonstrated low sulphur abundance and should be enriched with this nutrient to ensure that the plants would produce yields adequate in terms of both quality and quantity.

Key words: arylsulphatase activity, sulphur, soil, macroelements.

AKTYWNOŚĆ ARYLOSULFATAZY ORAZ ZAWARTOŚĆ SIARKI OGÓŁEM I JEJ FORM POD WPLYWEM NAWOŻENIA AZOTEM I POZOSTAŁYMI MAKROELEMENTAMI

Abstrakt

Celem pracy było określenie wpływu mineralnego nawożenia na aktywność arylosulfatazy (EC 3.1.6.1.) oraz zawartość siarki ogółem i jej frakcji w glebie spod uprawy kukurydzy. Próbkę gleby pobrano z doświadczenia polowego, założonego na terenie RZD w Grabowie nad Wisłą przez IUNG w Puławach, dwukrotnie w sezonie wegetacyjnym kukurydzy w 2008 roku. W doświadczeniu zastosowano tylko nawożenie mineralne: wzrastające dawki saletry amonowej (0, 50, 100, 150, 200 kg N ha⁻¹) oraz nawożenie: P – 80 kg P₂O₅ ha⁻¹, K – 140 kg K₂O ha⁻¹, Ca – 200 kg CaO ha⁻¹, Mg – 70 kg MgO ha⁻¹ i S w formach nawozowych z innymi pierwiastkami. Aktywność arylosulfatazy oznaczono wg metody Tabatabai i Bremnera, a siarkę ogółem i siarczanową zgodnie z metodą Bardsleya-Lancastera. Nawożenie saletrą amonową wpływało na koncentrację siarki organicznej i siarczanowej oraz aktywność arylosulfatazy w glebie płowej. Zawartość siarki ogółem wynosiła średnio 0,053 g kg⁻¹ i nie zależała od zastosowanego nawożenia mineralnego. Optymalną dawką azotu, po której zastosowaniu stwierdzono w glebie największą aktywność arylosulfatazy, było 100 kg N ha⁻¹. Na podstawie zawartości siarki siarczanowej (średnio 11.0 mg kg⁻¹) zakwalifikowano badaną glebę do gleb o średniej zasobności w siarkę, dlatego uprawa kukurydzy wymaga uzupełnienia zawartości tego pierwiastka, by umożliwić roślinom wydanie plonu o odpowiedniej jakości i wielkości.

Słowa kluczowe: arylosulfataza, siarka, gleba, makroskładniki.

INTRODUCTION

Over the last twenty years, sulphur deficiency has been recognized as a constraint on crop production all over the world (ERIKSEN et al. 2004, MAS-CAGNI et al. 2008, SCHERER 2009). The main reasons are the reduction of sulphur emission from various industrial sources, increasing use of high-analysis low-S-containing fertilizers, and the decreasing use of S-containing fungicides and pesticides (SCHERER 2001, ERIKSEN et al. 2004). Sulphur in soil occurs in inorganic and organic forms and it is cycled between these forms by mobilization, mineralization, immobilization, oxidation and reduction processes. Organic sulphur occurs mainly in two primary forms: as ester sulphates and carbon bonded (C-S). While organic sulphur compounds are largely immobile, inorganic sulphur is more mobile and sulphate (SO₄²⁻) is the most mobile form (SCHERER 2001). Arylsulphatases are enzymes that catalyse the hydrolysis of aromatic sulphate esters (C-O-SO₃⁻) to phenols (R-OH) and sulphate (SO₄²⁻). In soil, sulphate esters represent a large fraction (25.3-93.1%) of the total S and therefore, arylsulphatases may be important for mobilisation of inorganic SO₄²⁻ for plant nutrition (FITZGERALD 1976). Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (SINSABAUGH 1994). Mineral fertili-

sation stimulates biochemical reactions in soils, however, it is also a supplier of anions an excessive concentration of which can lead to the inhibition of enzymatic reactions in soil.

The aim of the paper was to determine the effect of mineral fertilisation on the activity of arylsulphatase and the content of total sulphur and its fraction in soil under maize cultivation.

MATERIAL AND METHODS

The soil was collected from a field experiment set up in the Agricultural Experimental Station in Grabów nad Wisłą, conducted by the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy. The soil sampling was performed twice in the vegetative period of maize in 2008. The soil was a very good rye complex soil. The experiment involved only mineral fertilisation consisting of increasing rates of ammonium nitrate (0, 40, 80, 120, 160 kg N ha⁻¹), and fertilisation with P – 80 kg P₂O₅ ha⁻¹, K – 140 kg K₂O ha⁻¹, Ca – 200 kg CaO ha⁻¹, Mg – 70 kg MgO ha⁻¹ and S in fertilisation forms with other elements. The experiment was performed as a split-plot design, the first factor involved increasing rates of nitrogen fertiliser, and the second one corresponded to fertilisation with the other macroelements. There were six treatments of the second factor. The first level involved a fertiliser combination of all the minerals, and then one element was absent in the following treatments; hence, the following six treatments:

1. P, K, Ca, Mg, S;
2. – , K, Ca, Mg, S;
3. P, – , Ca, Mg, S;
4. P, K, – , Mg, S;
5. P, K, Ca, – , S;
6. P, K, Ca, Mg, – .

In the treatments with S, single superphosphate and potassium sulphate were used, while the treatments without S consisted of triple superphosphate and high-percentage potassium salt.

The activity of arylsulphatase was determined following the TABATABAI and BREMNER method (1970), and the sulphate sulphur activity was analyzed according to the BARDSLEY-LANCASTER method (1960). Organic sulphur was calculated from the difference between the content of total sulphur and S-SO₄²⁻. The results of the sulphur content and the activity of the examined enzyme were processed using an analysis of variance with Tukey's semi-intervals of a confidence test ($p = 0.05$).

RESULTS AND DISCUSSION

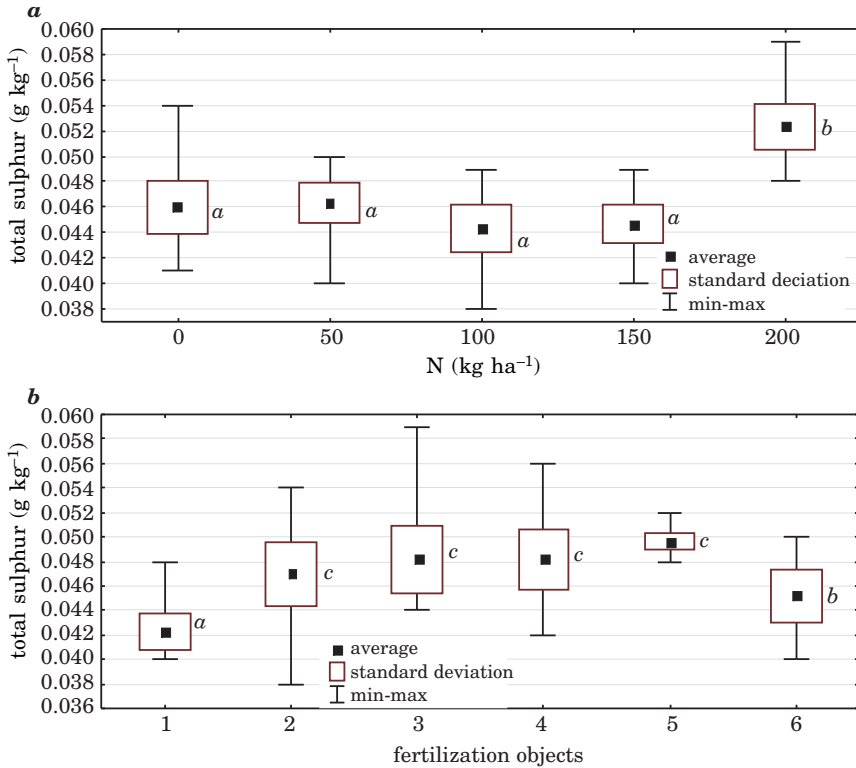
The reaction of the soil collected from the field in Grabów nad Wisłą ranged from 6.18 to 6.81 pH (Table 1). The reaction of the analysed soil ranged from slightly acid to neutral. Changes in the soil reaction were caused by neither the increasing rates of ammonium nitrate nor the various combinations of P, K, Ca, Mg and S fertilisation. CZEKALA et al. (2002) found that changes in the soil reaction were to a greater extent caused by the crops grown than nitrogen fertilisation. The content of sulphur available to plants depends on the soil reaction; when exposed to a higher soil pH, more sulphur is released owing to a higher rate of organic matter decomposition; when the soil pH is lower, an increased adsorption of sulphates on hydrate iron and aluminium oxides as well as kaolinite occurs (JOHNSON 1984). Similarly, the activity of soil enzymes depends on the concentration of hydrogen ions in soil. Arylsulphatase has a broad pH optimum from 5.8-8.2 (KLOSE et al. 1999).

Table 1

Reaction (pH in 1 mol KCl dm⁻³) of soil in the year of the experiment

Treatments	Nitrogen fertilization (kg ha ⁻¹)				
	0	50	100	150	200
1. P, K, Ca, Mg, S	6.28	6.59	6.54	6.76	6.56
2. - , K, Ca, Mg, S	6.51	6.36	6.49	6.59	6.25
3. P, - , Ca, Mg, S	6.23	6.51	6.72	6.18	6.37
4. P, K, - , Mg, S	6.72	6.45	6.63	6.56	6.64
5. P, K, Ca, - , S	6.67	6.33	6.58	6.38	6.58
6. P, K, Ca, Mg, -	6.81	6.51	6.75	6.36	6.61

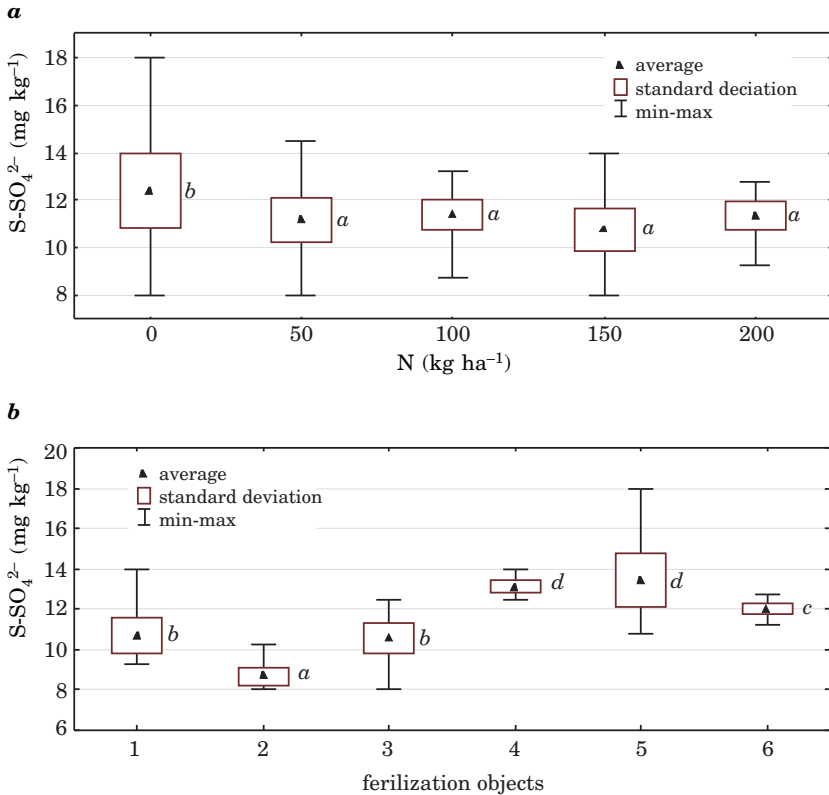
The content of total sulphur in soil ranged from 0.038 g kg⁻¹ to 0.059 g kg⁻¹. MOTOWICKA-TERELAK and TERELAK (1998) indicated that the content of S_{og} in mineral soils under agricultural use ranged from 0.07 to 1.07 g kg⁻¹. In the examined soil, the content of total sulphur was found below the range characteristic for mineral soils in Poland's agricultural areas. According to YANG et al. (2007) or KOTKOVA et al. (2008), the total soil sulphur depends on the type of fertilisers applied (mineral or organic). In our experiment, both types of fertilisation, with nitrogen and with the other macroelements, affected the content of sulphur in the soil. In the plots receiving either NK or NPKS fertilizers for more than 80 years, no significant accumulation of total sulphur, as compared with the control, could be detected (YANG et al. 2007). The highest total sulphur content was observed in the soil collected from the treatments with the highest rate of ammonium nitrate (200 kg ha⁻¹) – Figure 1. The other nitrogen fertiliser rates did not show any significant effect on the content of this macroelement in soil. However, fertilisa-



1. P, K, Ca, Mg, S; 2. -, K, Ca, Mg, S; 3. P, -, Ca, Mg, S; 4. P, K, -, Mg, S;
 5. P, K, Ca, -, S; 6. P, K, Ca, Mg, -
 a, b, c – homogenous groups are marked the same letter

Fig. 1. Content of total sulphur in soil as dependent on fertilization

tion with the other macroelements resulted in a significant effect of the type of macroelements on the content of total sulphur. Its highest rate was determined in soil fertilised with P, K, Ca, -, S (treatment 5) – Figure 2. The total sulphur content in soil from that treatment was 8% higher than in the control treatment without sulphur fertilisation and 14% higher than in the treatment including fertilisation with all the macroelements. The present research did not identify the effect of the soil sampling date on the content of this sulphur fraction. Total sulphur includes the total content of organic and mineral compounds of this element which occurs in soil and so the application of fertilisers containing sulphur results in an increase in the content of the nutrient in soil, whereas the immobilization and mineralization processes depend on the physicochemical soil properties, climate and the vegetation (McGRATH et al. 2003) and occur at varies intensity in the vegetation period (WIELEBSKI 2000).



1. P, K, Ca, Mg, S; 2. -, K, Ca, Mg, S; 3. P, -, Ca, Mg, S; 4. P, K, -, Mg, S;
5. P, K, Ca, -, S; 6. P, K, Ca, Mg, -

a, *b*, *c*, *d* – homogenous groups are marked the same letter

Fig. 2. Content of sulphate sulphur in soil as dependent on fertilization

Inorganic S is generally much less abundant than organically bound S in most of the agricultural soils (BOHN et al., 1986). Sulphate, the most common form of inorganic S, is divisible into SO_4^{2-} in soil solution, adsorbed SO_4^{2-} and mineral sulphur (SCHERER 2001). Sulphate sulphur in the analysed soil ranged from 8 to 18 mg kg^{-1} , depending on the fertilisation with nitrogen and the other nutrients (Figure 2). In most agriculturally used soils in Poland, the content of sulphate sulphur does not exceed 25 mg kg^{-1} of soil. In most soils, i.e. 70% of the total arable land, the content of this sulphur fraction ranges from 5.0 to 20.0 mg kg^{-1} (LIPINSKI et al. 2003). The content of sulphate sulphur determined in our study was, on average, 11 mg kg^{-1} . This level of soil richness in available sulphur is considered a medium one, hence maize needs supplementary sulphur fertilisation (LIPINSKI et al. 2003). The content of sulphate sulphur depended significantly on the rates of ammonium nitrate. The highest content of this sulphur form, 8% higher, was

reported in the soil sampled from the control treatments with no nitrogen fertilisation (Figure 2a). As for the fertilisation with macroelements, the highest content of sulphates occurred in the soil collected from the treatments fertilised with P, K, Mg, S (4) and P, K, Ca, –, S (5) – Figure 2b.

In Polish soils, organic sulphur is a dominant form of the element, with its content falling within a wide range of 6 to 688 mg kg⁻¹. The share of this sulphur fraction in total sulphur varies from 50 to 80% in the humus horizons of mineral soils (MOTOWICKA-TERELAK, TERELAK 1998). In the soil sampled from the Grabów experiment, the share of organic sulphur in total sulphur depended on the fertilisation variant, and ranged from 61 to 80% (Figure 3). The content of sulphur in organic compounds found in the soil

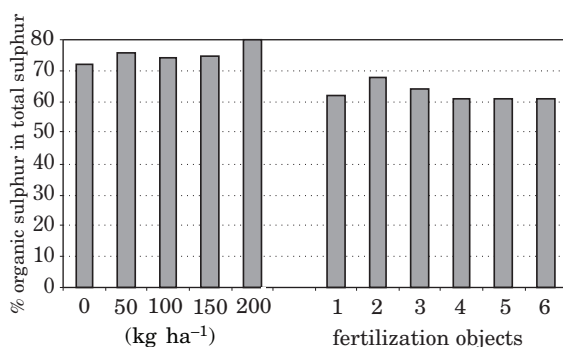
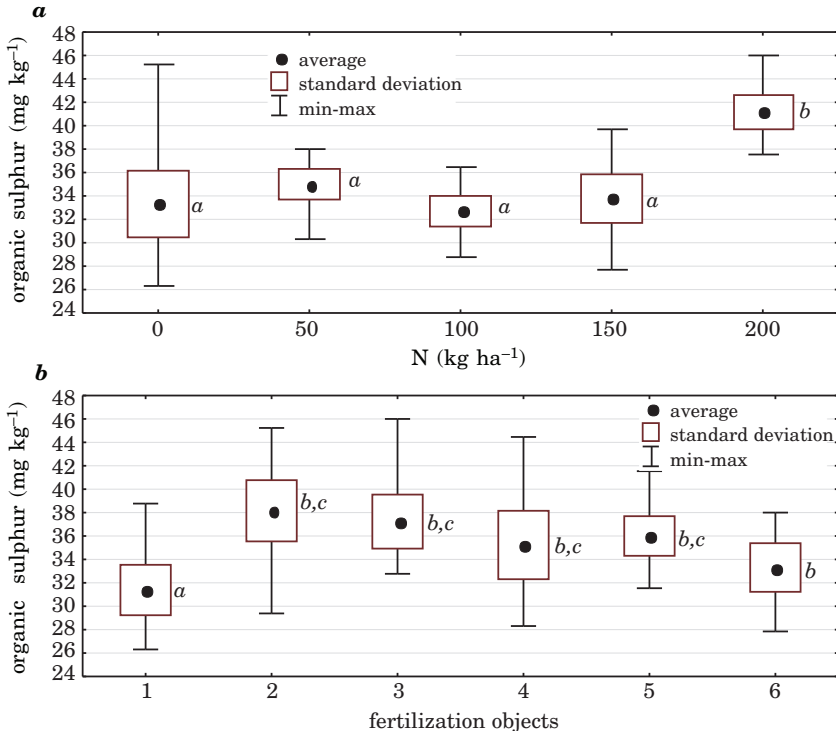


Fig. 3. Percentages of organics sulphur in total sulphur in soil as dependent on fertilization

was within the range from 26 to 46 mg kg⁻¹ (Figure 4) and did not undergo significant changes during the maize growing season, although it depended on the applied fertilisation. The highest amount of this sulphur fraction was noted in the treatments fertilised with the highest rate of ammonium nitrate and fertilisers containing –, K, Ca, Mg, S (treatment 2) and P, –, Ca, Mg, S (treatment 3) – Figure 5. KNIGHTS et al. (2001) found that a 153-year-long application of FYM in the experiment in Broadbalk resulted in an increase in the content of organic sulphur in soil, whereas soil fertilised with mineral fertilisers only showed a low level of organic sulphur in all plots. The authors also observed an increase in the level of mineralization in the soil sampled from the plots fertilised with FYM, as compared with those treated with mineral fertilisation and so they claim that long-term FYM fertilisation definitely supplies plants with sulphur.

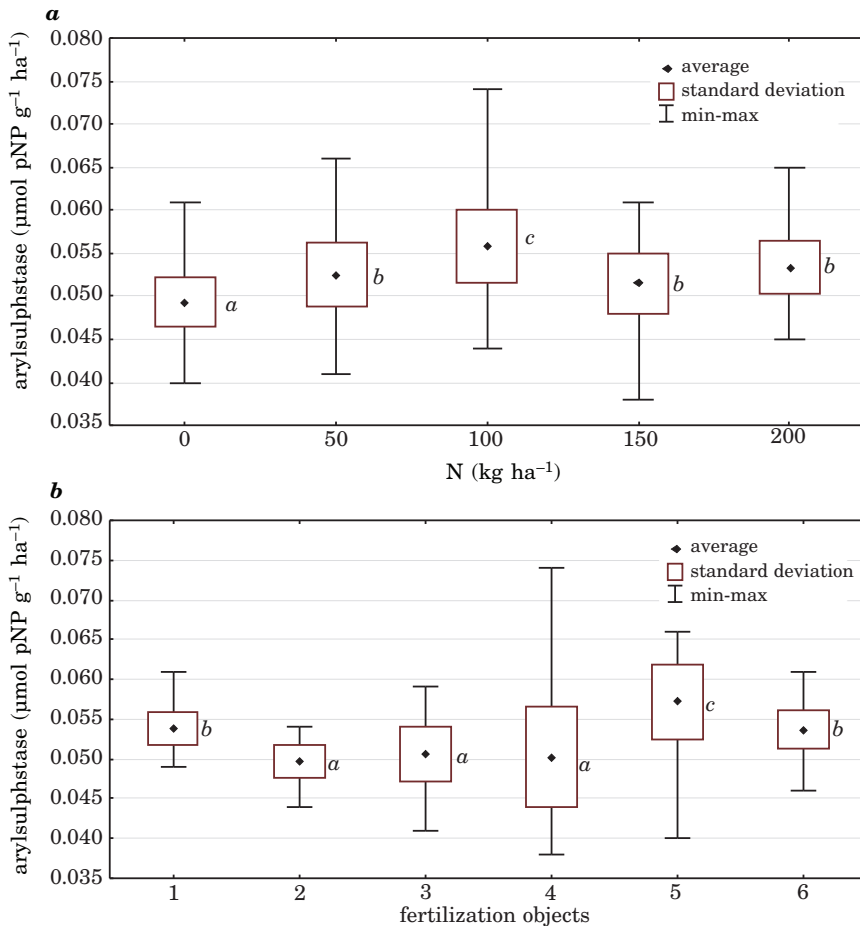
Soil enzymes play a biochemical function in the overall process of organic matter decomposition in the soil system (BURNS 1983). They are engaged in catalysing several important reactions necessary for the life processes of microorganisms in soils and the stabilisation of soil structure, the decomposition of organic waste, formation of organic matter and nutrient cycling (DICK et al. 1994). All soils contain enzymes that determine soil met-



1. P, K, Ca, Mg, S; 2. -, K, Ca, Mg, S; 3. P, -, Ca, Mg, S; 4. P, K, -, Mg, S;
 5. P, K, Ca, -, S; 6. P, K, Ca, Mg, -
 a, b, c – homogenous groups are marked the same letter

Fig. 4. Content of organic sulphur in soil as dependent on fertilization

abolic processes, which in turn depend on its physical, chemical, microbiological and biochemical properties (MAKOL, NDAKIDEMI 2008). The activity of arylsulphatase in the analysed Luvisol soil depended on the fertilisation, ranging from 0.038 to 0.066 $\mu\text{mol pNP g}^{-1} \text{h}^{-1}$. The optimal rate of ammonium nitrate which coincided with the highest activity of the hydrolase was 100 kg N ha⁻¹. In an experiment analysing soil from a field under potato fertilized with different doses of farmyard manure (0, 20, 40, 60 and 80 t ha⁻¹) and mineral nitrogen (0, 45, 90, 135 kg N ha⁻¹), the highest activity of arylsulphatase (an average 0.018 $\mu\text{mol pNP g}^{-1} \text{h}^{-1}$) was determined in the samples fertilised with nitrogen at the amount of 45 kg ha⁻¹ (SIWIK-ZIOMEK, KOPER 2010). Fertilisation with the other macronutrients affected the activity of arylsulphatase, which was the highest in samples of the soils fertilised with P, K, Ca, -, S (treatment 5) and all the macroelements applied in the experiment (treatment 1) and P, K, Ca, Mg, -, (treatment 6) – Figure 5. Literature reports (GANESHAMURTHY, NIELSEN 1990, GERMIDA et al. 1992) discuss a negative effect of ions (NO_3^- , NO_2^- , PO_4^{3-} , SO_4^{2-} , Cl^-) on the soil enzy-



1. P, K, Ca, Mg, S; 2. -, K, Ca, Mg, S; 3. P, -, Ca, Mg, S; 4. P, K, -, Mg, S;
 5. P, K, Ca, -, S; 6. P, K, Ca, Mg, -
 a, b, c - homogenous groups are marked the same letter

Fig. 5. Arylsulphatase activity in soil as dependent on fertilization

matic activity. The experiments made in Grabów on Haplic Luvisol did not reveal any inhibiting effect of mineral fertilisation on the activity of this enzyme. WYSZKOWSKA, KUCHARSKI, KUCHARSKI (2010) showed in a pot experiment that β -glucosidase, arylsulfatase and phosphatases were less resistant to copper contamination in cropped than in uncropped soil. The soil enzymatic activity undergoes complex biochemical processes, e.g. integrated and organic synthesis, immobilisation and stability of enzymes (KHAZIYEV, GULKE 1991).

In the current experiment, no significant coefficients of correlation were found between the content of total sulphur and its fractions versus the activity of arylsulphatase.

CONCLUSIONS

1. The effect of fertilisation with nitrogen and the other macroelements (P, K, Ca, Mg, S) on the content of total sulphur and its fraction and the activity of arylsulphatase was identified.

2. In respect of the sulphate sulphur content (an average of 11.0 mg kg⁻¹) the soil was classified as having an average sulphur content, and therefore must be supplemented for maize to achieve the right quality and quantity of yields.

3. The optimal rate of ammonium nitrate for which the highest activity of arylsulphatase was reported was 100 kg N ha⁻¹.

4. Low concentrations of total and organic sulphur in the soil fertilised with mineral fertilisers only would suggest that natural and organic fertiliser should be applied.

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