

# CHANGES IN THE CONTENT OF SULPHATE SULPHUR AND ARYLSULPHATASE ACTIVITY IN SOIL UNDER POTATO CAUSED BY FERTILIZATION

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## Abstract

Biological processes which shape soil fertility are affected by microorganisms and enzymes they produce as well as the rate of biogeochemical transformations in the cycling of elements. One of the enzymes is arylsulphatase (EC 3.1.6.1.), which hydrolyses sulphate esters with aromatic radical, releasing sulphate ions according to the equation:  $R-C-O-SO_3^- + H_2O \rightarrow R-C-OH + SO_4^{2-} + H^+$ . The enzyme plays an essential role in the sulphur cycle in soil and it can be an indicator of sulphur mineralization in soil. For his study, soil was sampled from a field under potato fertilized with different doses of farmyard manure (0, 20, 40, 60 and 80 t·ha<sup>-1</sup>) and mineral nitrogen (0, 45, 90, 135 kg N·ha<sup>-1</sup>). The activity of arylsulphatase was assayed according to Tabatabai and Bremner, while sulphate (VI) sulphur was determined as described by Bardsley and Lancaster. The content of organic carbon in the soil ranged from 8.168 to 10.96 g·kg<sup>-1</sup> and depended on FYM fertilization, while the content of total nitrogen ranged from 0.889 to 1.012 g·kg<sup>-1</sup> with an average of 0.960 g·kg<sup>-1</sup> for FYM and mineral nitrogen doses. The effect of fertilisation on changes in the amount of sulphate sulphur and the activity of arylsulphatase in the soil was noted. The content of sulphate sulphur throughout the research ranged from 21.49 to 24.83 g·kg<sup>-1</sup>. The higher the FYM doses, the higher the content of the fraction of sulphur available to plants. The soil provided a good supply of sulphur to plants. The activity of the enzyme ranged from 0.010 to 0.024 μM pNP·g<sup>-1</sup>·h<sup>-1</sup>. Its highest activity (an average 0.018 μM pNP g<sup>-1</sup>·h<sup>-1</sup>) was recorded in the samples fertilised with nitrogen at the amount of 45 kg·ha<sup>-1</sup>. Both parameters changed during the potato vegetation period.

**Key words:** arylsulphatase, fertilization, soil, sulphate sulphur (VI).

## OKREŚLENIE ZMIAN ZAWARTOŚCI SIARKI SIARCZANOWEJ I AKTYWNOŚCI ARYLOSULFATAZY W GLEBIE SPOD UPRAWY ZIEMNIAKA W ZALEŻNOŚCI OD NAWOŻENIA

### Abstrakt

Procesy biologiczne kształtujące żyzność gleby są związane m.in. z drobnoustrojami i wydzielanymi przez nie enzymami oraz tempem przemian biogeochemicznych w krążeniu pierwiastków. Arylosulfataza (EC 3.1.6.1.) jest enzymem, który hydrolizuje estry siarczanowe z rodnikiem aromatycznym, uwalniając jony siarczanowe, zgodnie z reakcją:  $R-C-O-SO_3^- + H_2O \rightarrow R-C-OH + SO_4^{2-} + H^+$ . Odgrywa on istotną rolę w obiegu siarki i może być wskaźnikiem mineralizacji jej związków w glebie. Celem pracy było zbadanie wpływu zróżnicowanych dawek obornika (0, 20, 40, 60 i 80 t·ha<sup>-1</sup>) i azotu mineralnego (0, 45, 90, 135 kg N·ha<sup>-1</sup>) na aktywność arylosulfatazy i zawartość siarki siarczanowej (VI) w glebie podczas uprawy ziemniaka. Aktywność arylosulfatazy oznaczono wg metody Tabatabai i Bremnera, a zawartości siarki siarczanowej (VI) wg metody Bardsleya-Lancastera. Zawartość węgla organicznego w badanej glebie mieściła się w zakresie 8,168-10,96 g·kg<sup>-1</sup> i zależała od nawożenia obornikiem. Zawartość azotu ogółem wynosiła 0,889-1,012 g·kg<sup>-1</sup>, średnio 0,960 g·kg<sup>-1</sup>, dla dawek obornika i azotu mineralnego. Stwierdzono wpływ nawożenia na zmiany ilości siarki siarczanowej oraz aktywność arylosulfatazy w badanej glebie. Aktywność badanego enzymu wynosiła 0,010-0,024 μM pNP g<sup>-1</sup>·h<sup>-1</sup>. Najwyższą aktywność arylosulfatazy (średnio 0,018 μM pNP g<sup>-1</sup>·h<sup>-1</sup>) stwierdzono w próbkach nawożonych azotem w ilości 45 kg·ha<sup>-1</sup>. Zawartość siarki siarczanowej w glebie w okresie wegetacyjnym ziemniaka wynosiła 21,49-24,83 g·kg<sup>-1</sup>. Zwiększające się dawki obornika powodowały wzrost koncentracji frakcji siarki przyswajalnej dla roślin. Badaną glebę cechuje dobre zaopatrzenie roślin w siarkę. Aktywność arylosulfatazy i zawartość siarki siarczanowej (VI) w glebie zmieniały się w sezonie wegetacyjnym ziemniaka.

Słowa kluczowe: arylosulfataza, nawożenie, gleba, siarka siarczanowa (VI).

## INTRODUCTION

Biological processes which shape soil fertility are mainly associated with microorganisms and enzymes they excrete as well as the ratio of biogeochemical processes in elemental cycles. The level of soil enzymatic activity has been considered a sensitive index of its fertility and cultivation potential (MYSKÓW et al.1996). Organic and mineral fertilization introduces nutrients into soil and thus determines the development and activity of soil microorganisms. Soil sulphatases are mainly synthesized by bacteria and fungi. An example of a soil sulphatase is arylsulphatase (EC 3.1.6.1.), which hydrolyses sulphate esters with aromatic ring, releasing sulphate ions according to the equation  $R-C-O-SO_3^- + H_2O \rightarrow R-C-OH + SO_4^{2-} + H^+$ . This enzyme plays an important role in the cycling of sulphur and can be an indicator of the mineralization of sulphur compounds in soil (GERMIDA et al. 1992).

The objective of the study was to assess effects of fertilization with farmyard manure and mineral nitrogen applied in various doses on the content of sulphate sulphur (VI) and arylsulphatase activity in soil under potato.

## MATERIALS AND METHODS

Soil samples were collected from a long-term experiment carried out at the Grabowo Agricultural Experimental Station by the Department of Plant Nutrition and Fertilization of the Institute of Tillage, Fertilization and Soil Science in Puławy. Considering its fractional composition, the soil was classified as light clay (brown podzolic soil). The crop rotation was: potato, winter wheat, spring barley and clover. The sampling was done four times (10 April, 04 June, 20 July, 12 September 2004) during the vegetation period of potato (medium-early cv. Wiking). The fertilization consisted of farmyard manure (FYM) in the doses: 0, 20, 40, 60 and 80 t·ha<sup>-1</sup>, or mineral nitrogen as ammonium nitrate in the doses: N<sub>0</sub>– 0, N<sub>1</sub>– 45, N<sub>2</sub>– 90, and N<sub>3</sub>– 135 kg N·ha<sup>-1</sup>. Arylsulphatase activity was assayed according to Tabatabai and Bremner (1970), while sulphate sulphur was measured as described by Bardley–Lancaster and modified by COMN-IUNG (1960). Concentrations of the other nutrients were determined by the commonly used methods (LITYŃSKI et al. 1976). The significance of the content of sulphate sulphur(VI) and activity of arylsulphatase was evaluated with the use of Tukey's half-zones of confidence ( $p=0.05$ ). Calculations were done using the FR-ANALWAR (Microsoft Excel) software.

## RESULTS AND DISCUSSION

The content of organic carbon in the soil ranged within 8.168-10.96 g·kg<sup>-1</sup> and depended on fertilization with FYM. The lowest concentration of this bioelement was found in soil samples collected from the control objects. As the rates of FYM rose, increasing accumulation of organic carbon in the investigated soil was noticed (Table 1). A similar effect of FYM on the content of organic carbon was observed earlier in many experiments by other authors, e.g. MERCIK et al. (1995). Concentration of total nitrogen was less differentiated and fluctuated within the range 0.889-1.012 g·kg<sup>-1</sup> (mean for FYM and mineral nitrogen doses – 0.960 g·kg<sup>-1</sup>) – Table 1. Small differences were found in the C:N ratio calculated for the samples under study. The highest value of this ratio (12) was observed for samples taken from plots fertilized with manure at the dose 60 t·ha<sup>-1</sup> and nitrogen at the dose N<sub>0</sub> (Table 1). These values indicated that the process of mineralization was stronger than nitrogen immobilization in the soil.

The values of pH measured in 1 mol·dm<sup>-3</sup> KCl ranged within 5.3-5.8 (Table 1), which meant that the soil was acidic or slightly acidic. The lowest pH was recorded for the soil fertilized with the highest dose of nitrogen (Table 1). It has been reported earlier that the soil reaction between 5.5 and 6.2 is best for arylsulphatase activity (TABATABAI, BREMNER 1970).

Table 1

The content of total organic carbon, total nitrogen, the C:N ratio and sulphate sulphur (VI) as well as activity of ariylsulphatase in soil and soil pH

FYM (I factor) (t·ha <sup>-1</sup> )	Nitrogen fertilization II factor (kg N·kg <sup>-1</sup> )													
	C (g·kg <sup>-1</sup> )						N (g·kg <sup>-1</sup> )						C:N	
	N <sub>0</sub>	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	$\bar{x}$	N <sub>0</sub>	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	$\bar{x}$	N <sub>0</sub>	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>
0	8.168	8.494	8.602	8.233	8.374	0.889	0.966	0.924	0.966	0.936	9	9	9	9
20	9.057	8.732	8.862	9.490	9.035	0.980	0.956	0.956	0.956	0.962	9	9	9	10
40	9.837	9.512	10.66	10.64	10.16	0.991	1.012	0.942	0.987	0.983	10	9	11	11
60	10.77	10.36	10.23	9.945	10.32	0.931	0.970	0.956	0.921	0.944	12	11	11	11
80	10.70	10.57	10.96	10.29	10.61	0.959	0.966	0.980	1.005	0.977	11	11	11	10
$\bar{x}$	9.705	9.533	9.863	9.720	9.705	0.950	0.974	0.951	0.967	0.960	-	-	-	-
LSD <sub>0,05</sub>	I - 0.435						II - n.s						II - n.s	
	SO <sub>4</sub> <sup>2-</sup> (mg·kg <sup>-1</sup> )						ariylsulphatase activity ( $\mu$ M pNP g <sup>-1</sup> ·h <sup>-1</sup> )						pH mol·dm <sup>-3</sup> KCl	
0	22.00	22.33	22.04	21.49	21.96	0.022	0.010	0.012	0.010	0.013	5.8	5.7	5.5	5.3
20	22.08	22.62	21.79	22.11	22.19	0.012	0.013	0.016	0.011	0.013	5.3	5.4	5.4	5.3
40	22.55	22.34	21.79	22.87	22.39	0.024	0.020	0.019	0.011	0.018	5.4	5.4	5.4	5.2
60	23.94	24.83	22.02	22.77	22.39	0.011	0.027	0.014	0.015	0.017	5.4	5.4	5.5	5.3
80	23.87	21.85	23.14	22.95	22.95	0.018	0.021	0.019	0.010	0.017	5.4	5.5	5.5	5.4
$\bar{x}$	22.89	22.79	22.20	22.44	22.58	0.017	0.018	0.016	0.011	0.016	-	-	-	-
LSD <sub>0,05</sub>	I - 0.571						I - n.s						II - 0.050	
	I/II - 1.082						I/II - 0.011						I/II - 0.011	

n.s. - non-significant

Concentrations of sulphate sulphur (VI) ranged within 21.49-24.3  $\text{mg}\cdot\text{kg}^{-1}$  and were significantly affected by fertilization, both with FYM and mineral nitrogen (Table 1). Most of Polish arable soils contain sulphur concentrations below 25  $\text{mg}\cdot\text{kg}^{-1}$  soil. About 70% of Polish arable land contain from 5 up to 20  $\text{mg}\cdot\text{kg}^{-1}$  of this fraction of sulphur (LIPINSKI et al. 2003). In our samples, we found on average 22.58  $\text{mg}$  sulphate sulphur (VI) per  $\text{kg}$  soil, which, in terms of soil abundance in sulphur, allows us to classify it as of a very high sulphur content. Therefore, a good supply of this element ( $\text{S}\text{-SO}_4^{2-}$ ) to plants seems ensured (LIPINSKI et al. 2003). An increase in the sulphate sulphur (VI) concentrations in brown podzolic soil was observed along with the increasing doses of farmyard manure (Table 1). However, fertilization with ammonium sulphate did not give such an unambiguous effect on this sulphur fraction. The highest concentration of  $\text{SO}_4^{2-}$  (22.89  $\text{mg}\cdot\text{kg}^{-1}$ ) was found in soil samples collected from plots without nitrogen fertilization, while the lowest amount (22.20  $\text{mg}\cdot\text{kg}^{-1}$ ) was recorded for the dose 95  $\text{kg N}\cdot\text{ha}^{-1}$  (Table 1).

The resources of soil sulphate sulphur (VI) fluctuated during the vegetation period of potato. The highest concentration of this fraction was noticed in soil samples collected on the third sampling date (25.14  $\text{mg}\cdot\text{kg}^{-1}$ ), while the corresponding amounts recorded on the first sampling date were lower by 22 % than these found in the samples taken in July (Figure 1).

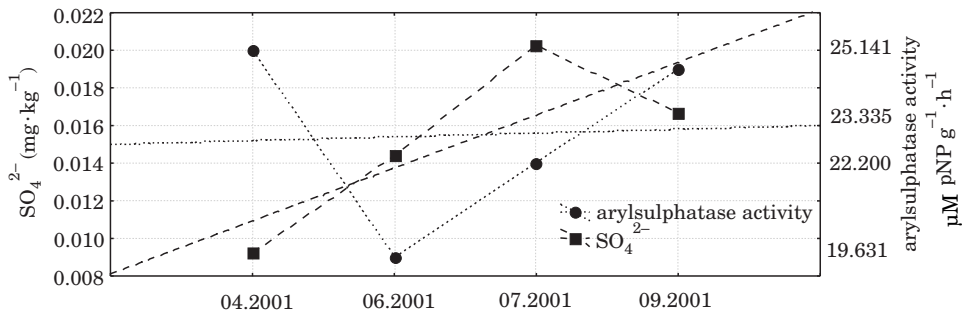


Fig. 1. Sulphate sulphur (VI) content and arylsulphatase activity in potato soil

The activity of arylsulphatase ranged from 0.010 to 0.024  $\mu\text{M pNP g}^{-1}\cdot\text{h}^{-1}$ . Fertilization with ammonium nitrate affected this parameter. The use of nitrogen fertilizer in the dose of 135  $\text{kg}\cdot\text{ha}^{-1}$  caused a decrease in arylsulphatase activity in the investigated soil. Its highest activity (mean 0.018  $\mu\text{M pNP g}^{-1}\cdot\text{h}^{-1}$ ) was observed in samples fertilized with nitrogen in the dose 45  $\text{kg}\cdot\text{ha}^{-1}$  (Table 1). Such results indicated an inhibitory effect of ammonium nitrate on the enzyme activity. A negative effect of some ions ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ) on soil enzymatic activity was reported by many other authors (DICK et al. 1988, GANESHAMURTHY, NIELSEN 1990, GERMIDA et al. 1992). Significantly dynamic changes in the activity of this enzyme

was observed in our study during the potato vegetation period. The highest activity of the enzyme was found at the beginning of this period, followed by a 22% decline in July and a gradual increase until the end of the vegetation of potato (Figure 1).

## CONCLUSIONS

1. The analyzed brown podzolic soil revealed a very high content of sulphur, which should ensure a very good supply of this bioelement to the plants included in the rotation of crops.

2. The highest activity of arylsulphatase and concentration of sulphate sulphur (VI) was found after fertilization with ammonium nitrate at the dose of  $45 \text{ kg} \cdot \text{ha}^{-1}$ .

3. A decrease in the enzyme activity accompanied by a high content of the sulphur fraction available to plants and increasing doses of ammonium nitrate suggested an inhibitory effect of sulphate (VI) and nitrate (V) ions on arylsulphatase activity in the investigated soil.

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