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B – zestawienie danych
C – analizy statystyczne
D – interpretacja wyników
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THE EFFECT OF SULPHUR-CONTAINING FERTILIZERS ON SOIL BIOLOGICAL PROPERTIES

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S u m m a r y

The aim of this paper was to determine the effect of sulphur-containing mineral fertilizers on the number of microorganisms and soil enzymatic activity. The research was carried out under conditions of a two-year field experiment. The effect of fertilization with sulphur in the sulphate form (ammonium sulphate and Saletrosan 26 makro) and in the elemental form (Wigor S) was analysed with respect to the control treatment without fertilization and to the treatment fertilized only with nitrogen, phosphorus and potassium. The effect of sulphur fertilizers was analysed at two levels of fertilization. In the first year of the experiment, spring rape was the test plant, and in the second year – winter wheat. The number of microorganisms (total number of bacteria, fungi and actinomycetes, as well as the number of sulphur bacteria) was determined by plate count method. Dehydrogenase and arylsulfatase activity was determined by colorimetric method, whereas catalase activity by manganometric method. During both years of the research, the number of microorganisms in the fertilized soil was greater than in the non-fertilized soil or these numbers were similar. Fertilization with a double dose of sulphur fertilizers had a stronger stimulating effect on the growth of fungi and actinomycetes than fertilization with a single dose. Compared to the soil fertilized with mineral fertilizers without sulphur, sulphur fertilization stimulated the growth of bacteria, fungi and actinomycetes especially after the first year of the research. The effect of fertilization on soil enzymatic activity was low.

Key words: *arylsulfatase, catalase, dehydrogenases, mineral fertilizers, number of microorganisms, sulphur*

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INTRODUCTION

In the conventional production system, yield maximization can be achieved through such agricultural practices as: intensive fertilization, using pesticides, mechanized cultivation, sprinkler irrigation etc. These practices condition the formation of favourable soil properties. Their incorrect execution may cause deterioration of soil physicochemical and biological properties [MIJANGOS *et al.* 2006], and even disturb functioning of entire ecosystems.

Soil biological properties are influenced by a number of factors (content of organic matter and nutrients, oxygenation, reaction, temperature, presence of pollutants) [STRĘK, TELESIŃSKI 2015; TELESIŃSKI *et al.* 2015; WŁODARCZYK *et al.* 2002]. Analysis of biological indicators such as the number of microorganisms, soil respiration and enzyme activity allows to assess soil fertility and productivity [BIELIŃSKA *et al.* 2000]. The presence of a positive correlation between soil biological activity and plant yielding also substantiates the use of soil enzyme determinations as tests for the assessment of soil fertility and productivity [MYŚKÓW *et al.* 1996]. According to NATYWA *et al.* [2014], microbiological indicators which are based on the number and activity of microorganisms in soil describe the state of the soil environment better and are more sensitive than the indicators which are based on physicochemical properties.

The aim of this paper was to determine the effect of sulphur-containing mineral fertilizers on the number of microorganisms and enzymatic activity of soil. The research was carried out under conditions of a two-year field experiment. The total number of bacteria, fungi and actinomycetes, the number of sulphur bacteria as well as the activity of dehydrogenase, catalase and arylsulfatase in the soil were determined.

RESEARCH METHODS

The experimental plot (N 50.091568, E 19.857655) was located at the experimental station of the University of Agriculture, located in Kraków-Mydlniki. The experiment was carried out in the years 2012–2013. The field experiment was established on typical brown soil classified as good wheat complex, soil quality class IIIb. It was soil with the granulometric composition of silt loam. The soil had a slightly acid reaction as well as a low content of sulphate sulphur and total sulphur (the assessment of sulphur abundance was conducted based on criteria adopted by the Institute of Soil Science and Plant Cultivation in Puławy [KABATA-PENDIAS *et al.* 1995]). Basic soil properties prior to establishing the field experiment are presented in Table 1.

Table 1. Soil parameters prior to establishing the field experiment

Tabela 1. Parametry gleby przed założeniem doświadczenia polowego

Parameter Parametr	Measurement unit Jednostka miary	Value Wartość
pH_{KCl}		5.96
Organic C C organiczny	$g \cdot kg^{-1}$ DM	9.82
Total N N ogółem	$g \cdot kg^{-1}$ s.m.	1.11
C : N		8.93
Available P P przyswajalny		123
Available K K przyswajalny	$mg \cdot kg^{-1}$ DM	109
Total S S ogółem	$mg \cdot kg^{-1}$ s.m.	180
Sulphate S S siarczanowa		12.6
N : S		6.30

Source: own study. Źródło: wyniki własne.

The experiment was conducted in randomized block design. It comprised 8 treatments (Tab. 2). Each treatment was carried out in four replications. The area of the experimental plot along with the protective belt was 880 m^2, the area of one plot (one replication) was 4 m^2 (the soil material was collected from 2.25 m^2). There were 2 m spacings between the plots.

Table 2. Treatments in the field experiment

Tabela 2. Obiekty nawozowe w doświadczeniu polowym

Treatment Obiekt	Applied fertilization (sulphur fertilizer form) Zastosowane nawożenie (forma nawozu siarkowego)
A	no fertilization (control) brak nawożenia (kontrola)
B	NPK
C	NPK + S1 (ammonium sulphate siarczan amonu)
D	NPK + S1 (Saletrosan 26 makro)
E	NPK + S1 (Wigor S)
F	NPK + S2 (ammonium sulphate siarczan amonu)
G	NPK + S2 (Saletrosan 26 makro)
H	NPK + S2 (Wigor S)

Explanations: S1 = single dose of sulphur, S2 = double dose of sulphur.

Wyjaśnienia: S1 = pojedyncza dawka siarki, S2 = podwójna dawka siarki.

Source: own study. Źródło: wyniki własne.

In the first year of the experiment, spring rape (*Brassica napus* L.), 'Markus' variety, was the test plant, whereas in the second year – winter wheat (*Triticum aestivum* L.), 'Wydma' variety. Doses of nutrients were adjusted to the requirements of the test plants (Tab. 3). The nutrients were supplied in the form of mineral

fertilizers. Nitrogen was applied in the form of ammonium nitrate (34% N) as well as in the form of ammonium sulphate (21% N) and Saletrosan 26 makro (26% N) in treatments C and F as well as D and G, respectively; dose of nitrogen in nitrogen-sulphur fertilizers was equalized with ammonium nitrate. Phosphorus was applied in the form of enriched superphosphate (40% P_2O_5), whereas potassium was applied in the form of potassium salt (60% K_2O). Sulphur was introduced in the form of three mineral fertilizers. Two of them, ammonium sulphate (24% S) and Saletrosan 26 makro (13% S), contain sulphur in sulphate form, whereas the third fertilizer, Wigor S, contains sulphur in elementary form (90% S). In each year of the field experiment, mineral fertilizers were applied once, before sowing. Only nitrogen dose was divided into two parts (the second dose was applied during the rape rosette stage and at the beginning of the winter wheat shooting stage, introducing ammonium nitrate to the soil of all fertilized treatments).

Table 3. Nutrient doses in the field experiment

Tabela 3. Dawki składników pokarmowych w doświadczeniu polowym

Test plant Roślina testowa	Dose, kg·ha^{-1} Dawka, kg·ha^{-1}				
	N[1)]	P_2O_5/P	K_2O/K	S1	S2
Spring rape Rzepak jary	100 + 40	75/32.7	160/132.8	25	50
Winter wheat Pszenica ozima	80 + 40	60/26.2	140/116.2	12.5	25

[1)] Fertilization at two dates. Explanations as under Table 1.

[1)] Nawożenie podzielone na dwa terminy. Objaśnienia jak w tabeli 1.

Source: own study. Źródło: wyniki własne.

Soil material was collected in each year of the experiment, in a representative manner (from a 0–20 cm layer, using Egner's sampler), after harvesting the test plant. Biological properties of the soil were determined in fresh material, and the results were converted into absolutely dry matter of the soil (in order to determine the content of absolutely dry matter, the soil was dried at 105°C until reaching constant weight).

Determination of the number of microorganisms (total number of bacteria, fungi and actinomycetes, as well as the number of sulphur bacteria) was carried out by pour plate method, with solid media. Meat infusion agar (for mesophilic bacteria), wort agar (for fungi), Gauss medium (for actinomycetes) and Postgate medium (for sulphur bacteria) were used [ATLAS, PARKS 1997; GHAZY *et al.* 2011; OLAŃCZUK-NEYMAN 1998]).

Dehydrogenase activity was determined by colorimetric method (BRZEZIŃSKA and WŁODARCZYK [2006] after CASSIDY *et al.* [1964]). The soil was incubated for 24 hours at 37°C with a colourless, water-soluble substrate, 2,3,5-triphenyltetrazolium chloride (TTC), which is enzymatically reduced to colourful, water-insoluble triphenylformazane (TPF). The generated product of the enzymatic reaction was

extracted with methyl alcohol. TPF content was determined by colorimetry (λ = 485 nm) using Beckman DU 640 UV/VIS spectrophotometer.

Catalase activity was determined by manganometric method (BRZEZIŃSKA and WŁODARCZYK [2006] after JOHNSON and TEMPLE [1964]). Reaction of the water suspension of soil with hydrogen peroxide solution 0.3% was conducted at room temperature (30 rot.·min^{-1}, 20 min). Excessive hydrogen peroxide was titrated with an aqueous solution of potassium tetraoxomanganate(VII) in the environment of sulphuric acid.

Arylsulfatase activity was determined by colorimetric method [TABATABAI, BREMNER 1970]. Concentration of p-nitrophenol (pNF), formed during incubation of soil with p-nitrophenyl sulphate (1 hour, 37°C), was determined by colorimetric measurement at a wavelength of 400 nm using Beckman DU 640 UV/VIS spectrophotometer.

Results of determinations of soil enzymatic activity were subjected to a statistical analysis; a univariate analysis of variance was performed. Significance of variance in mean values was determined using Duncan's test, at the significance level of $\alpha \leq 0.05$. Statistica 10 (StatSoft, Inc.) was used for statistical elaboration of the results.

RESULTS AND DISCUSSION

During the entire experiment, the number of bacteria in the fertilized soil remained at a level close to the one determined in the non-fertilized soil (control), or was higher (Fig. 1). After the first year of the research, the greatest number of bacteria (23–26% more than in the control soil) was found in the soil fertilized with

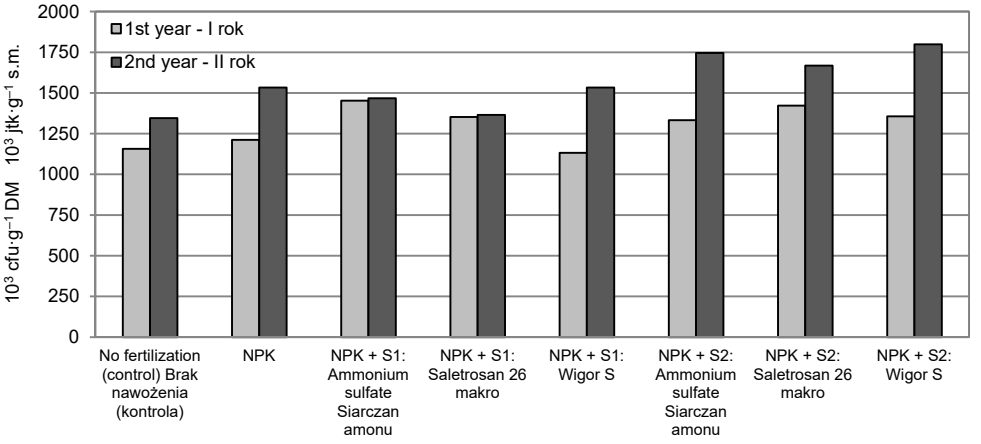

Fig. 1. Number of bacteria in soil; S1, S2 as in Table 3; source: own study

Rys. 1. Liczba bakterii w glebie; S1, S2 jak w tab. 3; źródło: wyniki własne

a single dose of ammonium sulphate and a double dose of Saletrosan 26 makro, whereas after the second year – in the soil fertilized with a double dose of all three sulphur fertilizers (24–34% more than in the control soil). Compared to soil fertilized with mineral fertilizers without sulphur, sulphur fertilization stimulated the growth of bacteria especially after the first year of the research.

The fertilized soil contained more fungi than the non-fertilized soil, especially after the first year of the research (the number of fungi increased by 32–132%) (Fig. 2). Fertilization with a double dose of sulphur had a greater stimulating effect on the growth of fungi than fertilization with a single dose, regardless of the type of sulphur fertilizer applied. After the first year of the research, the soil fertilized with sulphur contained considerably more fungi than the soil fertilized with mineral fertilizers without sulphur.

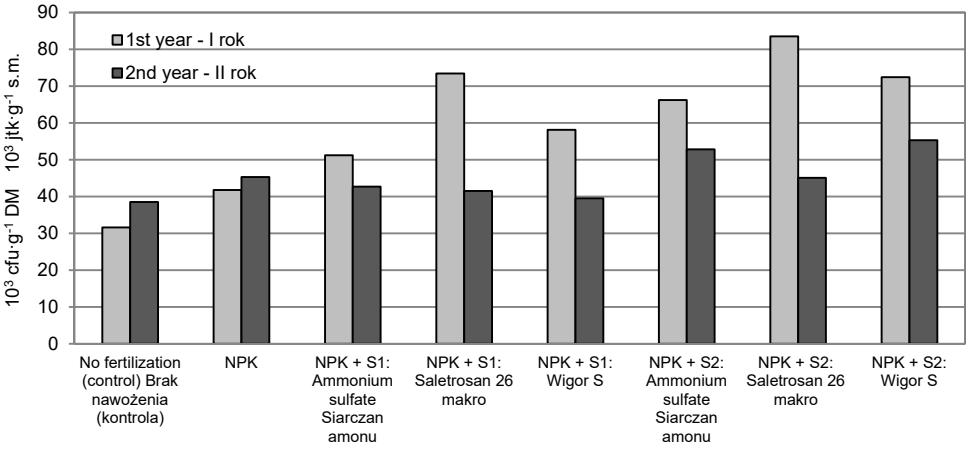

Fig. 2. Number of fungi in soil; S1, S2 as in Table 3; source: own study

Rys. 2. Liczba grzybów w glebie; S1, S2 jak w tab. 3; źródło: wyniki własne

Stimulation of the growth of actinomycetes as a result of fertilization was observed especially after the second year of the research (the fertilized soil contained 26–58% more actinomycetes than the control soil), but a positive effect of sulphur (in relation to fertilization with mineral fertilizers without sulphur) was observed mainly after the first year of the research (Fig. 3). Increasing the dose of all sulphur fertilizers led to a more intensified multiplication of this group of microorganisms in soil compared with the soil fertilized with a single dose of sulphur fertilizers.

After both years of the research, the soil fertilized with sulphur contained more sulphur bacteria than the non-fertilized soil and soil fertilized with mineral fertilizers without sulphur (Fig. 4). The greatest number of sulphur bacteria after the first year of the research was found in the soil fertilized with a single dose of ammonium sulphate and a double dose of Saletrosan 26 makro (93–116% more than in the

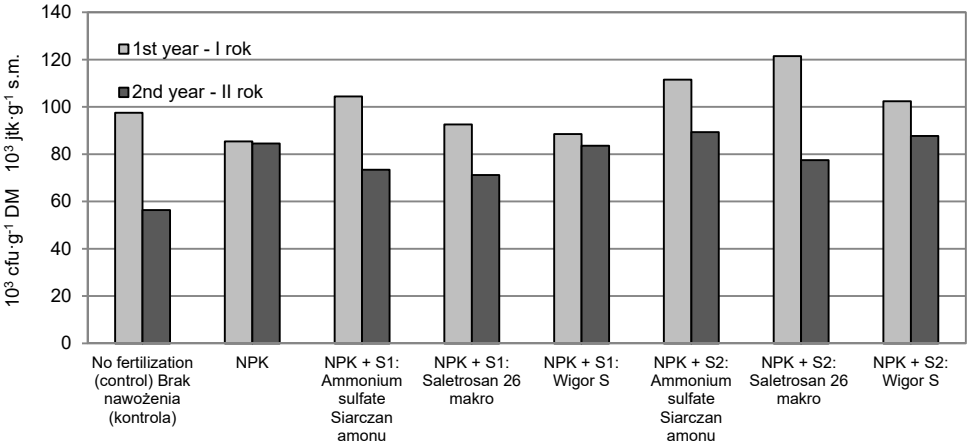

Fig. 3. Number of actinomycetes in soil; S1, S2 as in Table 3; source: own study

Rys. 3. Liczba promieniowców w glebie; S1, S2 jak w tab. 3; źródło: wyniki własne

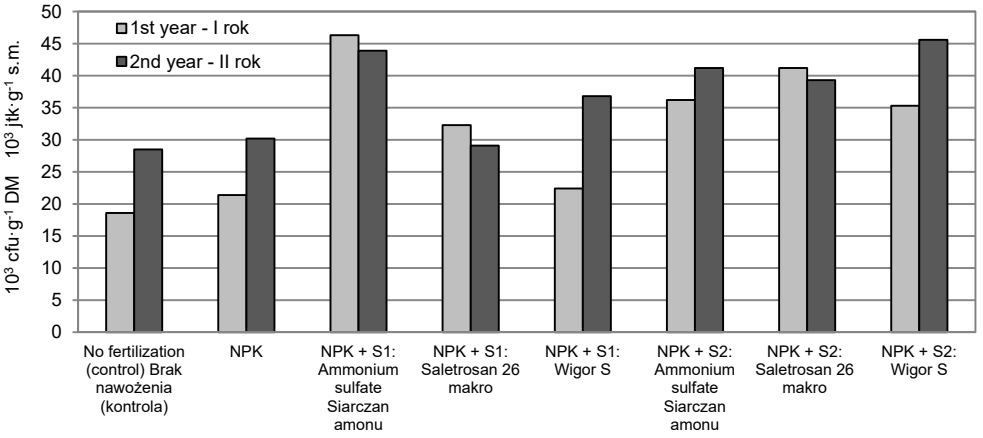

Fig. 4. Number of sulphur bacteria in soil; S1, S2 as in Table 3; source: own study

Rys. 4. Liczba bakterii siarkowych w glebie; S1, S2 jak w tab. 3; źródło: wyniki własne

soil fertilized with mineral fertilizers without sulphur). The greatest number of sulphur bacteria after the second year of the research was observed in the soil fertilized with a single dose of ammonium sulphate and a double dose of Wigor S (45–51% more than in the soil fertilized only with NPK).

After the first year of the research, no statistically significant effect of fertilization on dehydrogenase and catalase activity in the soil was observed (Tab. 4). After the second year of the research, dehydrogenase activity in the soil fertilized with mineral fertilizers without sulphur was much lower (by 18%) than in the control soil. Other types of fertilization did not significantly differentiate the dihydrogen-

Table 4. Dehydrogenase activity (μg TPF·kg^{-1} DM·h^{-1}), catalase activity (μmol H_2O_2·g^{-1} DM·min^{-1}), and arylsulfatase activity (μg pNF·g^{-1} DM·h^{-1}) in soil

Tabela 4. Aktywność dehydrogenaz (μg TPF·kg^{-1} s.m.·h^{-1}), katalazy (μmol H_2O_2·g^{-1} s.m.·min^{-1}) i arylosulfatazy (μg pNF·g^{-1} s.m.·h^{-1}) w glebie

Treatment Obiekt	Dehydrogenase activity Aktywność dehydrogenaz		Catalase activity Aktywność katalazy		Arylsulfatase activity Aktywność arylosulfatazy	
	1st year I rok	2nd year II rok	1st year I rok	2nd year II rok	1st year I rok	2nd year II rok
No fertilization (control) Brak nawożenia (kontrola)	57.2 a	61.9 b	8.15 a	6.54 b	21.9 c	25.3 b
NPK	68.7 a	50.6 a	7.57 a	5.97 ab	18.0 b	20.8 ab
NPK + S1 (ammonium sulphate) NPK + S1 (siarczan amonu)	53.9 a	62.6 b	6.67 a	5.76 a	13.6 a	20.0 a
NPK + S1 (Saletrosan 26 makro)	64.6 a	59.7 b	7.46 a	6.27 ab	19.0 bc	20.9 ab
NPK + S1 (Wigor S)	59.2 a	67.5 b	7.73 a	5.90 ab	18.6 b	21.1 ab
NPK + S2 (ammonium sulphate) NPK + S2 (siarczan amonu)	52.7 a	62.4 b	6.85 a	5.93 ab	12.5 a	19.1 a
NPK + S2 (Saletrosan 26 makro)	61.0 a	65.2 b	7.93 a	5.73 a	20.1 bc	21.6 ab
NPK + S2 (Wigor S)	52.0 a	66.8 b	6.92 a	5.77 a	15.0 a	22.3 ab

Explanations: S1, S2 as in Table 3; mean values in the columns marked with the same letters do not differ statistically significantly at the significance level $\alpha \leq 0.05$, according to Duncan's test.

Objaśnienia: S1, S2 jak w Tabeli 3; wartości średnie w kolumnach oznaczone tymi samymi literami nie różnią się istotnie statystycznie przy poziomie istotności $\alpha \leq 0{,}05$, według testu Duncana.

Source: own study. Źródło: wyniki własne.

ase activity in the soil. Catalase activity in the fertilized soil was not much diversified after the second year of the research. At the same time, catalase activity in the soil fertilized with a single dose of sulphur in the form of ammonium sulphate as well as a double dose in the form of Saletrosan 26 makro and Wigor S was significantly lower (by 12%) than the activity of the control soil.

After the first year of the research, arylsulfatase activity in the soil fertilized with mineral fertilizers without sulphur and also in the soil fertilized with both doses of ammonium sulphate and Wigor S was statistically significantly lower than the activity determined in the control soil, and the activity was reduced by 15–43% (Tab. 4). Fertilization with Saletrosan 26 makro did not have a significant effect on arylsulfatase activity in the soil. After the second year of the experiment, the soil fertilized with both doses of ammonium sulphate had a lower arylsulfatase activity (by 18–24%) than the non-fertilized soil. Other types of fertilization did not significantly differentiate the arylsulfatase activity in the soil.

In our research, we did not find any distinct negative effect of mineral fertilization on the number of microorganisms in the soil (Figs. 1–4). The positive effect of mineral fertilization on the number of microorganisms may result, among other

things, from soil enrichment in minerals. The increase in yield of fertilized plants (in other words, a greater weight of post-harvest residues in soil of fertilized treatments) is also beneficial [NATYWA *et al.* 2014]. On the other hand, large doses of mineral fertilizers may not stimulate the number and activity of microorganisms (even more so if it is unilateral fertilization) [NATYWA *et al.* 2010]. Fertilization with nitrogen fertilizers may lead to formation of toxic nitrosamines in soil [BARABASZ *et al.* 2002].

Determination of the activity of dehydrogenases, which occur in soil as an integral part of intact cells, is an indicator of intensity of respiratory metabolism of microorganisms, mainly bacteria and actinomycetes [PRAVEEN-KUMAR, TARAFDAR 2003], and thereby a very good indicator for determining soil biological activity. Catalase is an enzyme which breaks down hydrogen dioxide (compound with strong oxidizing properties); it has a protective role in cells. In our research, the effect of fertilization on dehydrogenase and catalase activity in the soil was insignificant (Tab. 4). ZAKARAUSKAITĖ *et al.* [2008] observed inhibition of the dehydrogenase activity as a result of long-term application of mineral fertilizers, whereas LIANG *et al.* [2014] showed no negative effect. The reduction of dehydrogenase activity in soil may result, among other things, from deficiency of carbon substrates which are susceptible to break down and from reduction of pH of the environment [GARCIA-GIL *et al.* 2000]. Our research showed a significant negative correlation between soil acidification and activity of all three studied enzymes (Tab. 5).

Table 5. Values of the correlation coefficient between selected properties of soil and its enzymatic activity

Tabela 5. Wartości współczynnika korelacji pomiędzy wybranymi właściwościami gleby a jej aktywnością enzymatyczną

Parameter Parametr	Dehydrogenase activity Aktywność dehydrogenaz	Catalase activity Aktywność katalazy	Arylsulfatase activity Aktywność arylosulfatazy
Catalase activity Aktywność katalazy	0.010		
Arylsulfatase activity Aktywność arylosulfatazy	0.372**	–0.065	
$[H^+]$	–0.385**	–0.232	–0.532***
Hydrolytic acidity Kwasowość hydrolityczna	–0.338**	–0.303*	0.537***
Sum of alkaline cations Suma kationow zasadowych	–0.060	0.853***	–0.190
Organic C C organiczny	0.183	0.407***	–0.179
Total N N ogółem	–0.043	0.598***	–0.480***
Total S S ogółem	0.148	0.125	0.446***
Sulphate S S siarczanowa	–0.147	0.582***	–0.453***

Explanations: * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

Objaśnienia: * istotny, gdy $p < 0{,}05$; ** istotny, gdy $p < 0{,}01$; *** istotny, gdy $p < 0{,}001$.

Source: own study. Źródło: wyniki własne.

Arylsulfatase plays an important role in processes of mineralization of organic sulphur and making it available to plants, carrying out hydrolysis of sulphate esters by splitting the O-S-bond: R -O-SO_3^- + H_2O —> ROH +H + SO_4^{2-}. It is assumed that 40–70% (50%, on average) organic sulphur in soil occurs in the form of sulphate esters [FRENEY, WILLIAMS 1983]. Our research showed a strong positive correlation between arylsulfatase activity and total sulphur content as well as a strong negative correlation between the activity of the enzyme and sulphate sulphur content in the soil (Tab. 5), which is consistent with the above-described role of the enzyme in circulation of sulphur in the environment. Arylsulfatase activity in samples of the fertilized soil was generally not higher than the activity in samples of the control soil. It is indicative of an inhibiting effect of the applied mineral fertilization on arylsulfatase activity, which SIWIK-ZIOMEK and KOPER [2008] also showed in their research. In her field experiment, SIWIK-ZIOMEK [2005] observed the highest arylsulfatase activity in soil collected in early spring from the treatment where full organic-mineral fertilization had been applied. CREGUT *et al.* [2009] prove that plants stimulate arylsulfatase activity, and arylsulfatase controls the supply of sulphate ions (which are necessary for the plant) through hydrolysis of sulphate esters that constitute a supply of sulphur relatively readily available in soil. When comparing arylsulfatase activity in soil under various crops, researchers prove that the activity of this enzyme increases along with the increase in plant requirements for sulphur [CREGUT *et al.* 2009; KNAUFF *et al.* 2003].

Along with plant cover, microorganisms determine the direction and nature of biochemical processes. They are also responsible for the formation of a certain type of equilibrium in the soil environment [DORAN *et al.* 1996]. There is no natural homeostasis in the soils of agrocenoses, so the main goal of contemporary systems of management should be to maintain arable soils at the optimum equilibrium [MIJANGOS *et al.* 2006; NATYWA *et al.* 2014]. In the research which formed the basis for this paper, the applied fertilization caused little changes in the activity of the studied soil enzymes compared with the enzymatic activity of the control soil. There was no evidence that the fertilizers, which had the most favourable effect on the number of microorganisms, stimulated the enzyme activity to the greatest degree. It was established that mineral fertilization, despite the fact that it stimulated multiplication of bacteria and actinomycetes in the soil (which points to a positive effect on soil biological properties), did not have a beneficial effect on the activity of soil enzymes. Such results are in accordance with the opinion that mineral fertilization enhances soil enzymatic activity to a lesser degree than organic fertilization and often leads even to distinct restriction of enzyme activity [KUCHARSKI 1997].

CONCLUSIONS

1. During both years of the field experiment, the number of microorganisms (bacteria – including sulphur bacteria, fungi and actinomycetes) in the fertilized soil was greater than in the non-fertilized soil or these numbers were similar. Fertilization with a double dose of sulphur fertilizers had a stronger stimulating effect on the growth of fungi and actinomycetes than fertilization with a single dose. Compared to soil fertilized with mineral fertilizers without sulphur, sulphur fertilization stimulated the growth of bacteria, fungi and actinomycetes especially after the first year of the research.

2. The effect of fertilization on soil enzymatic activity (dehydrogenase, catalase and arylsulfatase activity) was small.

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WPŁYW NAWOZÓW MINERALNYCH ZAWIERAJĄCYCH SIARKĘ NA BIOLOGICZNE WŁAŚCIWOŚCI GLEBY

Słowa kluczowe: *arylosulfataza, dehydrogenazy, katalaza, liczebność drobnoustrojów, nawozy mineralne, siarka*

S t r e s z c z e n i e

Celem pracy było określenie wpływu nawozów mineralnych zawierających siarkę na liczebność drobnoustrojów i aktywność enzymatyczną gleby. Badania prowadzono w warunkach dwuletniego doświadczenia polowego. Wpływ nawożenia siarką w formie siarczanowej (pod postacią siarczanu amonu i Saletrosanu 26 makro) i elementarnej (w formie Wigoru S) analizowano w odniesieniu do obiektu kontrolnego bez nawożenia oraz obiektu z nawożeniem jedynie azotem, fosforem i potasem. Działanie nawozów siarkowych analizowano na dwóch poziomach nawożenia. W pierwszym roku doświadczenia rośliną testową był rzepak jary, a w drugim roku pszenica ozima. Liczebność drobnoustrojów (ogólnej liczby bakterii, grzybów i promieniowców oraz liczby bakterii siarkowych) oznaczono metodą płytkową. Aktywność dehydrogenaz i arylosulfatazy oznaczono metodą kolorymetryczną, natomiast aktywność katalazy metodą manganometryczną. W ciągu obu lat badań liczebność drobnoustrojów w glebie nawożonej była większa niż w glebie nienawożonej lub liczebności te były zbliżone. Nawożenie podwójną dawką nawozów siarkowych silniej stymulowało rozwój grzybów i promieniowców niż nawożenie pojedynczą dawką. W porównaniu do gleby nawożonej nawozami mineralnymi bez siarki, nawożenie siarką stymulowało rozwój bakterii, grzybów i promieniowców zwłaszcza po pierwszym roku badań. Wpływ nawożenia na aktywność enzymatyczną gleby był mały.

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