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CONTENTS OF SULPHUR, TOTAL PROTEIN, METHIONINE AND CYSTEINE IN SPRING WHEAT BIOMASS AFTER FERTILIZATION WITH SEWAGE SLUDGE

ZAWARTOŚĆ SIARKI, BIAŁKA OGÓLNEGO, METIONINY I CYSTEINY W BIOMASIE PSZENICY JAREJ PO NAWOŻENIU OSADAMI ŚCIEKOWYMI

Abstract: The aim of conducted experiments was determining the effect of fertilization with municipal sewage sludge on the contents of sulphur, total protein, methionine and cysteine in spring wheat biomass. The research was conducted in a three-year field experiment on Stagnic Gleysol soil with granulometric composition of heavy silt loam.

On the basis of obtained results it was found that sulphur contents in wheat grain and straw on the treatments where fertilization was applied did not differ significantly. Applied fertilization with mineral fertilizers and farmyard manure and sewage sludge did not lead to any marked changes in weighted average contents of methionine and cysteine in spring wheat grain.

Keywords: spring wheat, sewage sludge, sulphur, total protein, methionine, cysteine

Adequate amount of nutrients supplied to plants and ensured optimal soil conditions are the main element determining production of expected yield of satisfactory biological and technological value. Wheat grain quality is to the greatest extent determined genetically, but the environmental and agrotechnological conditions, including fertilization, are also very important.

A comparison of the effect of natural and organic fertilizers with mineral fertilizer activity shows a greater influence of mineral fertilization on chemical composition of plants. When organic materials of waste origin, among others sewage sludge, are used for fertilization, one may expect their beneficial influence not only on the crop yield but also on its biological value [1].

Under soil and climatic conditions of Poland nitrogen is the element, which among the fertilizer components decisively influences the quantity and quality of obtained

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yields [2]. However, soil fertility and therefore its productivity is determined also by the content of bioavailable forms of other nutrients, including sulphur. The necessity to fertilize plants with sulphur is due in the first place to this element participation in nitrogen metabolism, which is better utilized at sufficient plant supply in sulphur [3]. The share of this element in forming such amino acids as methionine and cysteine, which are crucial components of proteins, is no less important.

The investigations were conducted to assess the content of sulphur, total protein, methionine and cysteine in spring wheat biomass after its fertilization with municipal sewage sludge.

Material and methods

The assessment of applied fertilization effect on the contents of sulphur, total protein, methinone and cysteine in spring wheat biomass was conducted in a three-year field experiment on ploughland situated 10 km west of Krakow (49°59.625′N; 19°41.910′E). The soil from the area of the experiment was classified as Stagnic Gleysol with granulometric composition of heavy silt clay. Before the experiment outset the basic soil properties were determined using the methods commonly applied by agricultural chemistry [4] and the obtained values have been presented in Table 1.

Table 1

Same properties of farmyard manure and sewage sludge used in experiment and soil before establishment of experiment

Property	Unit	FYM	SSI	SSII	Soil
pH H_2O	[-]	8.23	6.23	6.57	5.94
Dry matter	$[g \cdot kg^{-1}]$	226	297	258	—
Total N	$[g \cdot kg^{-1}$ d.m.]	34.0	26.2	41.6	1.59
Total P		12.8	8.2	22.3	72*
Total K		21.8	1.92	1.26	298*
Total S		4.76	9.66	6.96	0.41

* Content of available forms $[mg \cdot kg^{-1}$ d.m.].

The experiment was set up using randomized blocks methods and the area of fertilized plots was 30 m^2 (5 m · 6 m). The experimental design comprised 6 treatments in four replications: non-fertilized soil (0), soil fertilized with mineral fertilizers (NPK), soil fertilized with mineral fertilizers with sulphur supplement (NPKS), soil fertilized with pig *farmyard manure* (FYM), soil fertilized with municipal sewage sludge from mechanical-biological treatment plant in Krzeszowice (SSI) and soil fertilized with municipal sewage sludge from biological treatment plant in Czernichow (SSII). The sewage sludge and farmyard manure were applied once in the first year of the experiment. Basic chemical properties were determined in the sewage sludge and farmyard manure [5] and the obtained results were shown in Table 1.

Prior to the experiment outset the field was limed to make the soil pH meet the requirements stated in the regulation on municipal sewage sludge [6]. The liming was conducted using hydrated lime, according to 0.5 of hydrolytic acidity. The following year in spring, after completing basic cultivation measures farmyard manure and sewage sludge were spread on the surface of the plots and ploughed (to the depth of 20 cm). Supplementary doses of mineral fertilizers which were used two weeks later were mixed with the soil by means of aggregate cultivator and harrow. Nitrogen dose supplied with the fertilizer was 110.0 kg N $\cdot$ kg^{-1}. Phosphorus and potassium were supplemented to equal level supplied with fertilization on all treatments (except the control), phosphorus to 58.6 kg P $\cdot$ ha^{-1} as a single superphosphate and potassium to 120.0 kg K $\cdot$ ha^{-1} as 60 % potassium salt. Because of a considerable influence of sulphur on biological value of crop yield and great amounts of this element supplied to the soil with farmyard manure and municipal sewage sludge, an additional treatment was introduced to the experimental design, in which sulphur fertilization was conducted against the background of mineral fertilization with nitrogen, phosphorus and potassium. Sulphur was supplied with ammonium sulphate dosed 20.0 kg S $\cdot$ ha^{-1}. On treatments where basic and supplementary doses of mineral fertilizers were used, nitrogen was supplied as ammonium nitrate.

In order to avoid species changeability under the conditions of the experiments, each year the test plant was spring wheat, 'Jagna' c.v. Assumed plant density was 485 plants per 1 m^2. In order to supplement N, P, K nutrients and S but only on the treatment where sulphur was supplied in a mineral form, in the second and third year of the investigations the identical doses were applied as in the first year, but exclusively as mineral fertilizers, N – ammonium nitrate, P – single superphosphate, K – 60 % potassium salt and S – as ammonium sulphate. Wheat vegetation period depended on the weather conditions and lasted 117 days in the first year, 106 days in the second and 107 days in the third. Wheat was harvested at full maturity. During vegetation chemical treatment was applied to protect the plantation against weeds and fungal diseases. In order to determine wheat grain yield under field conditions, the plants were gathered from the 4 m^2 area of each plot separately. After ear threshing, the obtained grain and straw yields were dried (at 70 ^{o}C) and dry mass content was determined. In dried and milled plant material sulphur content was assessed after a sample mineralization in concentrated nitric acid [7], with ICP-AES method on JY 238 Ultrace apparatus. Methionine and cysteine concentrations were assessed after oxidating hydrolysis with formic acid followed by sample hydrolysis with 6 mol $\cdot$ dm^{-3} HCl solution conducted by means of ninhydrin method on Ingos AAA-400 analyzer.

Chemical analysis of the experimental material was conducted in four replications, while initial materials (farmyard manure, sewage sludge and soil) were analyzed in two replications and a reference sample NCS DC 733448 (China National Analysis Center for Iron & Steel) was attached to each analytic series.

A two-way analysis of variance (factors: fertilization x years) was conducted on the results obtained for grain and straw yields, whereas one-way ANOVA was conducted for sulphur, protein and amino acid contents (factor: fertilization) in totally randomized design using f-Fisher test. The significance of differences between arithmetic means was verified basing on the homogenous groups determined by t-Tukey test on significance level $p < 0.05$. All statistical computations were made using Statistica PL package [8].

The weather conditions (rainfall and temperature) during the experiment were presented in Tables 2 and 3.

Table 2

Monthly and periodic precipitation totals in study years [mm]

Year	Month						Σ
	III; Mar.	IV; Apr.	V; May	VI; Jun.	VII; Jul.	VIII; Aug.	Jan.; I – Dec.; XII
2005	20.7	49.1	61.3	40.6	113.4	102.6	597.5
2006	60.1	56.5	51.9	89.1	14.1	104.1	567.9
2007	61.1	15.4	51.7	72.1	71.0	76.4	830.4

Table 3

Mean daily air temperature in study years [^{o}C]

Year	Month						Mean
	III; Mar.	IV; Apr.	V; May	VI; Jun.	VII; Jul.	VIII; Aug.	Jan.; I – Dec.; XII
2005	–0.2	6.8	11.4	14.4	17.6	15.4	6.8
2006	0.2	5.6	10.9	15.0	18.6	15.6	7.5
2007	6.0	8.5	15.2	18.4	19.4	19.0	9.2

The weather course during the period of the experiment did not differ between the years. The highest rainfall amount (total from March to August) characterized the year 2005 (1^{st} year of the experiment). A higher annual temperature was registered in 2007, whereas average temperature during spring wheat vegetation (March to August) generally did not differ.

Results and discussion

Spring wheat grain yields registered in the experiment on the fertilized treatments in the first year were generally lower in comparison with the yields obtained on the same treatments in two subsequent years of the experiment (Table 4).

Table 4

Yield of grain spring wheat [$Mg \cdot ha^{-1}$ d.m. ± SD; n = 4]

Objects	1^{st} year	2^{nd} year	3^{rd} year
0	$2.53^{ab} \pm 0.15$	$1.93^{a} \pm 0.29$	$2.55^{ab} \pm 0.12$
NPK	$4.13^{bcde} \pm 0.54$	$4.81^{de} \pm 0.52$	$4.30^{de} \pm 0.40$
NPKS	$4.05^{bcde} \pm 1.01$	$4.05^{bcde} \pm 1.10$	$5.07^{e} \pm 0.28$
FYM	$3.36^{abcd} \pm 0.19$	$4.01^{bcde} \pm 1.16$	$5.39^{e} \pm 0.36$
SSI	$3.83^{bcde} \pm 0.32$	$4.83^{de} \pm 0.62$	$4.70^{de} \pm 0.44$
SSII	$3.40^{abcd} \pm 0.92$	$4.60^{de} \pm 0.98$	$4.42^{de} \pm 0.13$

Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Tukey test.

Additional sulphur fertilization in a mineral form (NPKS) did not cause any marked increase in wheat grain yield in comparison with the yields gathered on the treatments where farmyard manure (FYM), municipal sewage sludge (SSI, SSII) or nitrogen, phosphorus and potassium were applied in mineral form (NPK).

Greater diversification was noted for yields of spring wheat straw (Table 5).

Table 5

Yield of straw spring wheat [$Mg \cdot ha^{-1}$ d.m. ± SD; n = 4]

Objects	1st year	2nd year	3rd year
0	$3.01^{ab} \pm 0.56$	$2.03^{a} \pm 0.16$	$3.48^{b} \pm 0.30$
NPK	$4.91^{de} \pm 0.47$	$4.64^{cde} \pm 0.57$	$4.36^{cd} \pm 0.14$
NPKS	$5.65^{e} \pm 0.99$	$4.38^{cd} \pm 0.66$	$4.71^{cde} \pm 0.41$
FYM	$4.21^{bcd} \pm 0.21$	$4.61^{cde} \pm 0.37$	$4.71^{cde} \pm 0.30$
SSI	$3.99^{bcd} \pm 0.28$	$4.67^{cde} \pm 0.69$	$4.65^{cde} \pm 0.14$
SSII	$3.86^{bcd} \pm 0.74$	$4.63^{cde} \pm 0.59$	$4.62^{cde} \pm 0.26$

Means followed by the same letters did not differ significantly at $p < 0.05$ according to the Tukey test.

Irrespective of applied fertilization significantly greatest straw yields were gathered in the first year of the experiment on the treatment where mineral fertilization with nitrogen, phosphorus and potassium and sulphur (NPKS) was conducted. In the two subsequent years straw yields on the treatments where only mineral fertilization was used (NPK, NPKS) were higher (from 5 % to 22 %) in comparison with yields from the same treatments harvested in the first year. On the treatments where wheat was fertilized with farmyard manure (FYM) or municipal sewage sludge (SSI, SSII) in the second and third year of the experiments between 9 % and 20 % increase in straw biomass yield was obtained in comparison with the amount of straw harvested on these treatments in the first year.

Proper and rational fertilization affects the amount and improves biological and technological value of plant yields [9]. Unfavourable production conditions in Poland compared with the adjacent countries pose a severe obstacle in generating high yields of good quality. However, it is possible to improve soil fertility through proper agro-technical measures, including fertilization. According to Kwiatkowski et al [9] application of intensive cultivation measures on winter wheat plantations, irrespective of cultivar, allowed to reach over 72 % higher grain yields in comparison with yields from protected crops tended in a limited way. According to Lipa [10] limited mineral fertilization and lower herbicide doses may cause a decline in yields in result of worsening of most cereal stand and yield structure elements (number of ears per m^2, TGW). In the presented experiments, grain or straw yields obtained on the treatments where fertilization with farmyard manure (FYM) or municipal sewage sludge (SSI, SSII) was conducted and approximate to these produced on mineral fertilizer treatments were obtained in the second year of the experiment. It shows that also the kind of applied fertilization has a significant influence on plant yielding. Apart from agro-technical measures, the important factor limiting yielding of both winter and spring wheat forms is soil pH [11, 12].

No matter which fertilization was applied, sulphur concentrations both in the vegetative (straw) and generative (grain) parts of wheat were significantly higher than this element content assessed in wheat biomass from the non-fertilized treatment – (0) (Table 6). No marked differences were registered between sulphur content in wheat biomass fertilized with this element mineral form and S content in the biomass from the treatments where farmyard manure (FYM) or municipal sewage sludge (SSI, SSII) were used.

Table 6

Weighted mean (from three years) content of sulphur in biomass of spring wheat [$g \cdot kg^{-1}$ d.m. ± SD; n = 4]

Objects	Grain	Straw
0	$1.29^{a} \pm 0.06$	$0.92^{a} \pm 0.03$
NPK	$1.44^{b} \pm 0.04$	$1.12^{b} \pm 0.03$
NPKS	$1.41^{b} \pm 0.07$	$1.14^{b} \pm 0.05$
FYM	$1.44^{b} \pm 0.08$	$1.01^{ab} \pm 0.15$
SSI	$1.46^{b} \pm 0.02$	$1.12^{b} \pm 0.04$
SSII	$1.37^{ab} \pm 0.04$	$0.99^{ab} \pm 0.10$

Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to the Tukey test.

As reported by Kaczor et al [13] sulphur content depends on the plant development stage, its organ but also on fertilization. Increase in sulphur content resulting from fertilization with this element was noted by McGrath and Zhao [14] and Zhao et al [15]. It has been also reflected by the results of presented experiments. A diversification in sulphur contents was also noted between generative (grain) and vegetative (straw) wheat parts. Such dependency was not confirmed either by the Author's investigations or by Boreczek [16]. Despite the fact that wheat belongs to a group of plants with relatively low sulphur requirements, this element deficiency in soil may lead to lower nitrogen utilization [11].

Total (for three years) amounts of sulphur taken up with wheat grain and straw yield were on a similar level, whereas the differences between individual treatments were not statistically significant (Table 7).

Table 7

Total (from three years) uptake of sulphur with biomass of spring wheat [$kg \cdot ha^{-1}$ ± SD; n = 4]

Objects	Grain	Straw	Σ
0	$9.0^{a} \pm 0.6$	$7.8^{a} \pm 0.6$	$16.8^{a} \pm 0.4$
NPK	$19.0^{b} \pm 1.0$	$15.6^{bc} \pm 1.6$	$34.6^{b} \pm 2.4$
NPKS	$18.7^{b} \pm 3.0$	$16.9^{c} \pm 2.3$	$35.6^{b} \pm 4.9$
FYM	$18.4^{b} \pm 1.5$	$13.6^{bc} \pm 2.2$	$32.0^{b} \pm 2.3$
SSI	$19.5^{b} \pm 1.2$	$14.9^{bc} \pm 0.5$	$34.4^{b} \pm 0.9$
SSII	$17.0^{b} \pm 2.3$	$13.0^{b} \pm 2.1$	$30.0^{b} \pm 4.4$

Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to the Tukey test.

The highest amounts of sulphur (total amounts for grain and straw) were absorbed by wheat on the treatments where sulphur was supplied in mineral form (NPKS). In comparison with this treatment, sulphur quantities absorbed by wheat fertilized with farmyard manure or sewage sludge were between 3 % and 16 % lower.

Total protein content (N content multiplied by 6.25) in spring wheat grain from fertilized treatments was significantly higher than assessed in wheat grain on the treatment where no fertilization was applied (0) (Table 8). The lowest quantities of total protein were assessed in wheat grain receiving mineral fertilizers, without sulphur supplement (NPK) and in wheat grain from the treatment where farmyard manure (FYM) was used, however the results were not corroborated by the research conducted by Filipek et al [1]. On the treatments where wheat was fertilized with municipal sewage sludge (SSI and SSII), total protein contents were comparable with values assessed in wheat grain from the treatment on which mineral fertilization but with sulphur supplement (NPKS) was used. Total protein contents determined in spring wheat grain from these treatments were comparable to assessed by Filipek et al [1] in winter wheat grain fertilized with farmyard manure and sewage sludge from a dairy plant, but in only the limed series.

Table 8

Weighted mean (from three years) content of total protein [$g \cdot kg^{-1}$ d.m. ± SD; n = 4] and cysteine and methionine in proteins of grain of spring wheat [$g \cdot kg^{-1}$ proteins ± SD; n = 4]

Objects	Total protein	Cysteine	Methionine
0	$142.6^{a} \pm 4.13$	$12.7^{a} \pm 0.4$	$7.32^{a} \pm 0.28$
NPK	$158.2^{b} \pm 4.89$	$12.7^{a} \pm 0.5$	$7.11^{a} \pm 0.27$
NPKS	$160.8^{b} \pm 3.18$	$12.7^{a} \pm 0.4$	$7.16^{a} \pm 0.31$
FYM	$158.8^{b} \pm 8.04$	$12.2^{a} \pm 1.5$	$7.31^{a} \pm 0.69$
SSI	$161.2^{b} \pm 3.74$	$12.6^{a} \pm 0.7$	$7.59^{a} \pm 0.61$
SSII	$160.3^{b} \pm 3.51$	$12.4^{a} \pm 0.3$	$7.69^{a} \pm 0.24$

Means followed by the same letters in columns did not differ significantly at $p < 0.05$ according to the Tukey test.

According to Kwiatkowski et al [9] protein content in wheat grain is conditioned in the first place by the level of agrotechnical measures, by which not only optimal fertilization level is meant.

Cysteine content in spring wheat grain protein did not differ significantly between treatments (Table 8). Still, wheat grain protein from the treatments on which fertilization with farmyard manure (FYM) and municipal sewage sludge (SSI and SSII) was applied, contained between 0.8 % and almost 4 % less of this amino acid than the content assessed in wheat grain protein from the treatments where only mineral fertilizers (NPK, NPKS) were used, but also in comparison with the content assessed in grain protein on the treatment which was not receiving any fertilization (0).

An opposite dependence was observed for methionine content in grain protein. The content of this amino acid was the lowest in wheat grain protein from the treatments on which exclusively mineral fertilizers (NPK and NPKS) were applied. Methionine contents in wheat grain protein fertilized with farmyard manure (FYM) or municipal sewage sludge (SSI and SSII) were between 2.5 % and almost 8 % higher in comparison with the content assessed in grain protein on the treatments on which mineral fertilizers were applied.

Limited or unbalanced fertilization generally causes a decrease in plant yields, but also worsening of most elements of stand structure and quality of obtained biomass. This situation may be aggravated by increasingly frequent signals about among others sulphur deficiency in the soils of Europe, which significantly affects the improvement of biological properties of plant yields [17]. Fertilization with sewage sludge did not cause any marked changes in weighted average for three years cysteine and methionine content assessed in grain protein of wheat fertilized with mineral materials. According to Dubetz and Gardiner [18] the content of protein and amino acids change visibly under the influence of increasing nitrogen doses. In the Author's own investigations the level of nitrogen fertilization was not diversified, whereas the application of moderate dose of this element to the soil in mineral fertilizers and organic materials (farmyard manure, sewage sludge) contributed to stabilization of the content of studied amino acids in spring wheat grain protein.

Proper balancing of amino acids in feed doses with animal requirements is crucial not only for utilization of the most precious element, *ie* feed protein but also because of decreasing the amount of nitrogen excreted with urea in case of any amino acid excess in comparison with needs. The analyzed spring wheat grain, irrespective of applied fertilization, met the animal nutritional requirements with respect to methionine and cysteine contents [19].

Conclusions

1. No significant differences were registered between sulphur content in the biomass of wheat fertilized with this element mineral form and this element content in wheat biomass from the treatments where farmyard manure or sewage sludge were used as fertilizers.

2. Applied fertilization did not diversify total protein content in spring wheat grain.

3. Fertilization of spring wheat with municipal sewage sludge did not cause any notable changes of weighed average content of the analyzed amino acids.

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ZAWARTOŚĆ SIARKI, BIAŁKA OGÓLNEGO, METIONINY I CYSTEINY W BIOMASIE PSZENICY JAREJ PO NAWOŻENIU OSADAMI ŚCIEKOWYMI

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Abstrakt: Celem przeprowadzonych badań było określenie wpływu nawożenia komunalnymi osadami ściekowymi na zawartość siarki, białka ogólnego, metioniny i cysteiny w biomasie pszenicy jarej. Badania przeprowadzono w 3-letnim doświadczeniu polowym na glebie opadowo-glejowych o składzie granulometrycznym gliny ciężkiej pylastej.

Na podstawie uzyskanych wyników badań stwierdzono, że zawartość siarki w ziarnie i słomie pszenicy oraz zawartość białka w ziarnie z obiektów, w których zastosowano nawożenie nie różniła się istotnie. Zastosowane nawożenie nawozami mineralnymi, a także obornikiem i osadami ściekowymi nie spowodowało istotnych zmian w średniej ważonej zawartości metioniny i cysteiny w ziarnie pszenicy jarej.

Słowa kluczowe: siarka, pszenica jara, osady ściekowe, białko ogólne, metionina, cysteina