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CONTENT OF SELENIUM IN ALFALFA IN DEPENDENCE OF ITS DOSE AND THE TYPE OF SOIL

ZAWARTOŚĆ SELENU W LUCERNIE W ZALEŻNOŚCI OD JEGO DAWKI I TYPU GLEBY

Abstract: The aim of research was the assessment of the effect of selenium addition to soils of diversified granulometric composition (light loamy sand, light loam and clay silt) on the yielding and Se content in alfalfa harvested twice in the course of plant growing period. Selenium was introduced in the following doses: 0, 20, 40 and 60 $\mu g \cdot kg^{-1}$ of soil in the form of water solution of Na_2SeO_3.

The addition of selenium to light loamy sand in the amount of 20 and 40 $\mu g \cdot kg^{-1}$ of soil did not affect alfalfa yielding, while the dose of 60 $\mu g \cdot kg^{-1}$ resulted in decreased quantity of biomass harvested. Significantly higher yield, in comparison with the one obtained from control treatments and fertilized with the remaining Se doses, was determined for cultivation conditions involving light loam supplemented with 20 $\mu g \cdot kg^{-1}$ of this element and with 60 $\mu g \cdot kg^{-1}$ in the case of silty soil. Selenium content in plants increased according to gradually higher doses of this element, yet only on light loam supplemented with selenium dose of 60 $\mu g \cdot kg^{-1}$ it reached the level meeting animals' needs regarding this microelement.

The properties of the examined soils and introduced selenium doses differentiated availability of this element for plants. The higher quantity of selenium applied as fertilizer, the higher amount of this element passing into both extracts and Se-DTPA content was twice higher than that of Se-HCl. The content of selenium in alfalfa did significantly depend on total content of this component in the soil, as well as on the amount of DTPA and HCl (1mol $\cdot$ dm^{-3} concentration) – extracted Se forms.

Keywords: selenium, alfalfa, soil type

Investigation conducted recently proved that in the area of Poland a considerable share belongs to soils featuring low content of selenium [1–8]. Additionally, significant soil acidification can be the cause of poor uptake of this microelement by plants [9–13].

Selenium is classified to indispensable elements regarding animals feeding, especially carnivores [5, 10, 14]. Yet constantly maintained sale of feeds characterizing insufficient selenium content can lead to the occurrence of numerous diseases in

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animals, including diminished fertility, the increased number of miscarriages or food muscular dystrophy. High degree of selenium absorption from feeds and a slight difference between optimum and toxic quantity bring about the necessity of preceding any supplementation of this element by precise estimation of selenium resources in soils, also taking into account the factors conditioning its availability for plants and the ability of its accumulation featuring particular species [15].

The aim of the research was determination of selenium in alfalfa in relation to soil suitability classification and microelement dose applied.

Material and methods

The assessment of the effect of selenium dose on alfalfa yielding and accumulation of this element was based on the results of pot experiment established in pots of Wagner's type, whose volume amounted to 5.0 kg of air-dry soil. Investigation involved three types of soil, differing in their granulometric composition: *light loamy sand* (lls), *light loam* (ll) and *clay's silt* (cs). Selected soil properties were shown in Table 1.

Table 1

Selected properties of soils

Soil textural group	Share [%] of fraction < 0.02 mm	pH_{KCl}	CEC [cmol(+) · kg^{-1}]	org-C [g · kg^{-1}]	Se [μg · kg^{-1}]		
					Total	DTPA	HCl
Light loamy sand (lls)	12	3.7	4.4	12.5	92	6.0	7.5
Light loam (ll)	21	4.8	8.8	24.1	143	13.8	16.1
Clay's silt (cs)	41	6.7	27.0	23.5	180	16.0	17.5

CEC – *cation exchangeable capacity*; Se-DTPA and Se-HCl – selenium form extracted by DTPA (*Diethylene Triamine Pentaaceric Acid*) and 1 mol · dm^{-3} HCl solution, respectively.

Because of strongly acidic reaction of soil containing light loamy sand and acidic reaction of light soil, as far as its granulometric composition was concerned, the soils underwent liming with $CaCO_3$ in the dose being an equivalent of 1.0 hydrolytic acidity. After balancing soil moisture of all the samples to 60 % of their maximum water capacity, they were subjected to 14-day – incubation. Then there was applied basic mineral fertilization and selenium supplementation. Se was introduced in the form of water solution of Na_2SeO_3 in the doses of 0, 20, 40 and 60 μg Se · kg^{-1} d.m. of soil. The latter ones were determined so that the content of this element did not exceed the range assumed for soils belonging to particular granulometric groups, which was confirmed by earlier examination [6].

A test plant was alfalfa (*Medicago sativa*), 'Susi' cultivar. Plants biomass was harvested twice in the course of plant growing period, in the stage of flowering. All treatments were provided with the same mineral fertilization. Before sowing and after the first harvest of alfalfa there were applied the following compounds: 0.15 g N · kg^{-1} of soil (NH_4NO_3), 0.06 g P · kg^{-1} and 0.30 g K · kg^{-1} (K_2HPO_4 + KCl) and

0.03 g Mg · kg^{-1} ($MgSO_4 \cdot 7H_2O$). In both cultivation terms fertilization with phosphorus, potassium and magnesium was introduced twice, while nitrogen was used in three equal doses (each amounting 0.05 g N · kg^{-1} of soil). Harvested plant material, dried at room temperature was subjected to determination of selenium content by AAS method, accompanied by hydride generation using Spectr AA 220FS Varian device with VGA-76 attachment after the samples had been prepared according to methodic described in Annex to Ordinance by Ministry of Agriculture and Development of Rural Areas dated on 23rd January 2003 [16].

There was assayed total selenium content in soil samples collected after experiment had been completed and decomposed in the mixture of HNO_3 + HCl + H_2O_2 on water bath at the temperature of 95 ^oC, then subjected to selenium reduction to Se(IV) through the addition of 6 mol HCl · dm^{-3} [17]. Selenium form available for plants was determined after DTPA soil extraction with the use of Lindsay and Norwell method [18] and after extraction by 1 mol · dm^{-3} HCl solution. All determinations, both in plant and soil material, were done in three replications, separately for each pot. Obtained results were statistically elaborated with the use of analysis of variance and Tukey test, at significance level $p = 0.05$, according to Statistica program.

Results and discussion

Total selenium content in soils used for examination did not differ from the values reported by Piotrowska [19] and also by Patorczyk-Pytlik and Kulczycki [6] for those granulometric groups (Table 1). In comparison with the values obtained by the mentioned authors, the examined soils characterized low total selenium content.

The amount of selenium in light soil, according to the values reported by Gupta and Gupta [10], should be accepted as a critical one regarding the quality of harvested yield. However, according to criteria assumed by Zablocki [20] after Welles, even lower Se content than 300 μg · kg^{-1} should be treated as deficit from the point of view of feed value of the cultivated plants. Similarly to the results obtained by numerous authors [2, 6, 7, 19–21] it was possible to confirm significant relation between selenium content in the soil and the content of silt and clay fraction.

The content of DTPA- and HCl-extracted selenium forms was conditioned by granulometric composition of the examined soils (Table 1). From 6.7 % to 9.6 % of total Se content in soils passed to DTPA solution, so those values were slightly higher than the ones determined by Borowska and Koper [3]. In soils investigated by the authors mentioned above, DTPA-soluble selenium forms provided for approximately 6 %. The quantity of Se passing to 1 mol · dm^{-3} HCl solution was higher than that determined in DTPA, as it ranged from 7.5 μg · kg^{-1} (8.2 %) to 17.5 μg · kg^{-1} (11.2 %). In both solutions the most considerable share of soluble Se forms characterized light loam (ll) while light loamy sand featured the lowest percentage of soluble Se (lls).

Conducted research proved that both alfalfa yielding and selenium content in this plant was conditioned by the category of soil suitability classification, as well as the dose of selenium (Table 2).

Table 2

Yield and content and uptake of selenium by alfalfa

Dose [μg Se · kg^{-1}]	Yield [g d.m. per pot]			Content [μg Se · kg^{-1}]		Uptake [μg Se per pot]		
	harvest I	harvest II	Σ	harvest I	harvest II	harvest I	harvest II	Σ
Light loamy sand (lls)								
0	14.7	17.9	32.6	11.5	12.2	0.16	0.22	0.38
20	13.5	19.1	32.6	21.6	22.2	0.29	0.42	0.72
40	13.5	19.2	32.7	33.8	43.6	0.45	0.84	1.29
60	12.6	16.9	29.5	62.2	59.0	0.79	1.00	1.78
Mean	13.6	18.3	31.9	32.3	34.3	0.42	0.62	1.04
Light loam (ll)								
0	16.7	20.8	37.5	7.2	16.8	0.13	0.35	0.48
20	18.6	21.9	40.5	25.1	31.2	0.52	0.62	1.14
40	15.8	20.8	36.6	61.1	59.0	0.95	1.22	2.18
60	16.8	20.2	37.0	94.6	87.5	1.60	1.77	3.36
Mean	17.3	20.4	37.7	47.2	48.6	0.80	0.99	1.79
Clay's silt (cs)								
0	11.2	18.3	29.5	20.6	16.9	0.23	0.31	0.54
20	11.8	17.7	29.5	33.4	31.4	0.42	0.56	0.98
40	12.6	17.2	29.8	46.9	47.0	0.59	0.81	1.40
60	13.4	18.6	32.0	61.4	61.5	0.82	1.15	1.97
Mean	12.3	17.9	30.2	41.1	39.2	0.52	0.70	1.23
$LSD_{0.05}$ Interaction soil × doses	2.4	1.4	3.2	11.7	4.4	0.37	0.12	0.43

In both harvests alfalfa yield was significantly higher for alfalfa cultivation on medium-heavy soil than on the remaining soils. On all the soils markedly higher amount of alfalfa biomass, both from control treatments and from the treatments fertilized with selenium, was harvested in the second cutting. Introduction of 20 and 40 μg Se · kg^{-1} to light loamy sand (lls) did not diversify alfalfa yield size harvested in the first term, while it resulted in yield increase in the second cutting, as compared with the quantity of biomass obtained from control treatment. The highest Se dose (60 μg · kg^{-1}) in both terms resulted in the decrease in alfalfa biomass amount in relation to that harvested from control treatment, as well as to the treatments where lower doses of this element were applied.

Significantly higher biomass yield, as compared with control treatment and the treatments fertilized with the remaining Se doses, was recorded for medium-heavy soil (ll) under the influence of 20 μg Se · kg^{-1} and for heavy soil (cs) after introduction of 60 μg Se · kg^{-1}. Advantageous effect of low selenium doses on plants growth and development was also proved by Hartikainen et al [22], Xue et al [23] and Hawrylak-Nowak [24]. In plants classified as "not accumulating Se", high content of this element in their tissues do negatively affect synthesis of proteins [15], reduces starch content [25], increases phosphorus and calcium content and diminishes the quantity of potassium [24].

Selenium does not belong to chemical elements recognized as indispensable ones regarding growth and development of majority of cultivated plants, although when growing on soils featuring high Se content, plants can accumulate considerable amounts of this element [15, 22, 26, 27]. Positive significant correlation between total selenium content in soils and the quantity of this element in alfalfa was also confirmed by other authors [1, 2, 13, 20].

Trafikowska and Kuczynska [8] report that alfalfa harvested from cultivated fields in the region of Bydgoszcz, contained, on average, 34 μg Se $\cdot$ kg^{-1} d.m. Se contents obtained in our investigation point to the fact that alfalfa harvested from soils not fertilized with selenium contained lower amounts of this element (Table 2), as it ranged from 7.2 μg to 20.6 μg Se $\cdot$ kg^{-1} d.m. Judging from the point of view of animals nutrition, the mentioned Se amount should be regarded as deficit [5, 10, 14].

Applied selenium doses caused the increase in this microelement in aboveground parts of alfalfa cultivated on all the types of soils, yet Se content meeting optimum value, as far as animals nutrition is concerned, was recorded only in plants cultivated on light loam supplemented with 60 μg Se $\cdot$ kg^{-1} of soil.

In the first alfalfa harvest originating from control treatment significantly higher selenium content characterized plants cultivated on heavy soil (cs), while the lowest – on medium-heavy soil (ll). In plants coming from the second harvest, Se content in alfalfa cultivated on medium-heavy and heavy soil featured similar values. The latter ones were significantly higher than Se content determined in alfalfa growing on light soil. Similar relation involved alfalfa cultivated on soils supplemented with 20 μg Se $\cdot$ kg^{-1}. At higher doses of selenium (40 and 60 μg Se $\cdot$ kg^{-1}), in plants originating from both cuttings, the highest Se content was determined when plants were growing on medium-heavy soil (ll). This dependence was also observed for the quantity of selenium absorbed by alfalfa. Poorer ability of selenium uptake characterized plants originating from sandy soils, in comparison with those cultivated on loamy and silty soils, was also reported by Yläranta [21], Laser [12] and Dhillon et al [9], while Johnson [11] obtained reversed relation.

The properties of the examined soils, selenium dose and alterations this element underwent in soils in the course of plant growing period diversified solubility of Se forms in DTPA and 1 mol $\cdot$ dm^{-3} HCl solution (Table 3). In soil samples collected from control treatments the share of extracted Se forms was lower than that determined in the soils before experiment was established (Table 1). Evidently lower decrease in available Se forms, especially in light loamy sand, comparing with the remaining soils, indicates that intensive sorption process of introduced selenate(IV) does take place in these soils [26]. As the quantities of introduced selenium increase, there also increase the amount of this element passing to both extractors and the content of DTPA-extracted Se forms was approximately twice higher than that of 1 mol $\cdot$ dm^{-3} solution of HCl-extracted Se forms.

Table 3

Total selenium content and the content of Se forms extracted by DTPA and by 1 mol · dm^{-3} HCl solution in soils collected after alfalfa

Dose	Content of Se		Share of Se-DTPA* in total Se	Content of Se-HCl*	Share of Se-HCl in total Se
	Total	DTPA			
[μg Se · kg^{-1}]	[μg Se · kg^{-1}]		[%]	[μg Se · kg^{-1}]	[%]
Light loamy sand (lls)					
0	90	5.7	6.3	3.7	4.1
20	106	9.2	8.7	6.8	6.4
40	129	12.0	9.3	8.9	6.9
60	142	18.3	12.8	10.6	7.5
Mean	117	11.3	9.3	7.5	6.2
Light loam (ll)					
0	139	7.0	5.0	6.9	5.0
20	164	9.8	6.0	9.0	5.5
40	182	12.3	6.8	13.2	7.3
60	202	18.1	9.0	16.3	8.1
Mean	171	11.8	6.7	11.3	6.5
Clay's silt (cs)					
0	178	9.2	5.2	5.8	3.3
20	208	12.8	6.2	7.1	3.4
40	231	15.6	6.8	9.1	3.9
60	259	25.3	9.8	11.8	4.6
Mean	219	15.7	7.0	8.4	3.8
$LSD_{0.05}$ Interaction soil × doses	11.9	0.25		0.89	

Se-DTPA and Se-HCl – selenium form extracted by DTPA and HCl of 1 mol · dm^{-3} concentration, respectively.

Statistical analysis proved that selenium content in both alfalfa harvests and uptake of this elements by plants did significantly depend on total Se content in soils, as well as on the content of DTPA and HCl of concentration 1 mol · dm^{-3} – extracted selenium (Table 4).

Table 4

Correlation coefficients between Se content in alfalfa and its total content, as well as Se content extracted from soils by DTPA and HCl

Harvest	I harvest	II harvest	Total uptake
Total Se	0.55	0.53	0.49
Se-DTPA	0.79	0.80	0.69
Se-HCl	0.51	0.49	0.46

Significant at $p < 0.05$.

Conclusions

1. Introduction of 60 μg Se · kg^{-1} of soil did negatively affect the field of alfalfa cultivated on light loamy sand (lls), while it increased the quantity of alfalfa biomass originating from silty soil (cs). On light loamy soil, the best effect, expressed by alfalfa field size, was obtained from the treatment with Se supplemented soil in the dose of 20 μg · kg^{-1}.

2. As selenium dose increased, there also increased the content and amount of this microelement uptaken by alfalfa. The highest quantity of Se was absorbed by plants cultivated on light loamy soil, while the lowest uptake featured plants originating from light loamy sand.

3. Optimum feed value, regarding Se content, characterized alfalfa growing on light loamy soil fertilized with Se dose of 60 μg · kg^{-1}.

4. Selenium content in both alfalfa harvests did significantly depend on the properties of the soil used, including total Se content, as well as the amount of Se extracted by DTPA and HCl of 1 mol · dm^{-3} concentration.

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ZAWARTOŚĆ SELENU W LUCERNIE W ZALEŻNOŚCI OD JEGO DAWKI I TYPU GLEBY

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Abstrakt: Celem przeprowadzonych badań była ocena wpływu dodatku selenu do gleb o zróżnicowanym składzie granulometrycznym (piasku gliniastego lekkiego, gliny lekkiej oraz pyłu ilastego) na plonowanie oraz zawartość Se w lucernie, zbieranej dwukrotnie w okresie wegetacji. Selen zastosowano w dawkach: 0, 20, 40 i 60 μg · kg^{-1} gleby w postaci roztworu wodnego Na_2SeO_3.

Dodatek selenu do piasku gliniastego lekkiego w dawkach 20 i 40 μg · kg^{-1} gleby nie wpływał na plonowanie lucerny, natomiast 60 μg · kg^{-1} spowodowało obniżenie ilości zebranej biomasy. Istotnie większy plon niż określony dla roślin z obiektów kontrolnych oraz nawożonych pozostałymi dawkami stwierdzono w warunkach uprawy lucerny na glinie lekkiej pod wpływem 20 μg · kg^{-1}, a na glebie pylastej po zastosowaniu 60 μg · kg^{-1}. Zawartość selenu w roślinach zwiększała się wraz ze wzrostem dawki tego składnika, z tym że jedynie na glinie lekkiej z dodatkiem 60 μg · kg^{-1} kształtowała się ona na poziomie pokrywającym zapotrzebowanie zwierząt na ten mikroelement.

Właściwości użytych gleb oraz wielkość dawki selenu zróżnicowały dostępność tego pierwiastka dla roślin. Wraz ze wzrostem ilości selenu wprowadzonego do gleby zwiększała się ilość tego składnika przechodząca do obu ekstraktorów, z tym że zawartość Se-DTPA była około dwukrotnie większa niż Se-HCl. Zawartość Se w lucernie istotnie zależała od ogólnej zawartości tego pierwiastka w glebie, a także od zawartości form Se wyekstrahowanych przez DTPA oraz HCl o stężeniu 1 mol · dm^{-3}.

Słowa kluczowe: selen, lucerna, gatunek gleby