

THE EFFECT OF PLANT BIOSTIMULATION WITH 'PENTAKEEP V' AND NITROGEN FERTILIZATION ON THE CONTENT OF FOURTEEN ELEMENTS IN SPINACH

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Abstract. Foliar application of growth regulators or fertilizers containing biostimulators can influence the uptake and accumulation of mineral elements by plants. The aim of the research was to determine the influence of foliar application of 'Pentakeep V' and diverse nitrogen fertilization on the content of: Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V in spinach leaves. In 2006–2007 pot experiment with spinach *Spinacia oleracea* L. 'Spinaker F₁' cultivation on silty medium loam was carried out. The experiment design included two sub-blocks: with and without foliar nutrition. Plants with foliar nutrition were sprayed twice with 0.02% w/v 'Pentakeep V' fertilizer (3000 dm³ water per 1 ha). In each sub-block soil nitrogen fertilization (in the form of ammonium nitrate) was applied in following combinations: 1 – control (without N), 2 – 50% N dose prior to seed sowing (25 mg N·dm⁻³ of soil), 3 – 100% N dose prior to seed sowing (50 mg N·dm⁻³ of soil). Foliar application of 'Pentakeep V' resulted in (compared to not treated plants): a) significant reduction of the Ag, Al, Ba, Ga, Sr and Ti concentration in plants not fertilized with nitrogen, b) increase in Sr content in spinach fertilized with 100% of N dose, c) relatively weak tendency to lower V accumulation in control plants. The lowest concentration of cobalt was found in plants fertilized with 50% of N dose and not treated with 'Pentakeep V'. No interaction between foliar nutrition and nitrogen fertilization was found in reference to Cd, Cr, Li, Ni, Pb and Sb content in spinach plants.

Key words: foliar nutrition, mineral composition, heavy metals, trace elements

INTRODUCTION

Plant biostimulation (bioactivation, stimulation) can be carried out using organic and mineral compounds or its mixtures excluding nutrients and growth regulators [The Act of 10th July, 2007]. One of the most popular compound used in these agrotechnique is 5-aminolevulinic acid (ALA) included, among others, in Pentakeep[®] fertilizers.

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ALA is a common precursor to tetrapyrrole compounds found in chlorophyll an hemes, and is also a natural organic acid presented in all living organisms. ALA possesses physiological properties, such as increasing and keeping greening, promoting stomatal aperture opening. It can also contribute to improve resistance to stress under low-light, low-temperature and salt conditions, as well as reduce nitrate concentration [Tanaka et al. 2005]. A tight relation was found between ALA content in cells and photosynthetic activity in plants. [Memon et al. 2009, Tanaka et al. 2005, Yaronskaya et al. 2006].

Influence of exogenous ALA application into soil, nutrient medium or foliarly (in the form of pure compound or Pentakeep® fertilizers) on quantity and quality of crop yield has been relatively well documented [Babik et al. 2008, Ježek and Kopecký 2008, Nowak 2007, Tanaka et al. 2005]. Still, in publications available to the authors no information could be found on ALA interaction on heavy metal or trace element uptake by plants. Recognizing this aspect of ALA impact on mineral distribution in plants seems important as previous studies indicated that foliar application of growth regulators (e.g. benzyladenine [Smoleń 2008, Smoleń and Sady 2008]) or fertilizers containing biostimulators (Aminoplant syn. Siapton, Pentakeep V [Wierzbńska 2009]) significantly influenced uptake and accumulation of mineral elements by plants.

The aim of the research was to determine the influence of foliar application of 'Pentakeep V' along with diverse doses of nitrogen fertilization on the content of: Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V in spinach leaves.

MATERIAL AND METHODS

Spinach (*Spinacia oleracea* L.) 'Spinaker F₁' c.v. was cultivated in 2006–2007 in open-work containers sized 60×40×20 cm, placed in the open field under a shade providing fabric. The containers were filled with silty medium loam (35% sand, 28% silt and 37% clay – according to PTG PN-R-04033) with content of organic matter 2.44–2.52% (in 2006 and 2007) and the following concentration of the available nutrient forms soluble in 0.03 M acetic acid (respectively for 2006 and 2007): N (N-NO₃+N-NH₄) 16.6–86.3 mg, P 16.6–64.8 mg, K 37.6–53.1 mg, Mg 121.4–158.3 mg and Ca 1032.2–2342.9 mg in 1 dm³ soil. In 2006 and 2007 soil pH_(H₂O) was 6.38–6.99, while general concentration of salt in soil (EC) 0.19–0.41 mS cm⁻¹, respectively. The content of assimilable nutrient forms of phosphorus and potassium was supplemented before the cultivation to the following level: 60 mg P (in 2006) and 200 mg K dm⁻³ (in 2006 and 2007) of soil. When soil humidity was not sufficient, plants in containers were watered with municipal water.

The research comprised two sub-blocks with and without foliar application of 'Pentakeep V'. In the sub-block with foliar nutrition the plants were sprayed twice (on 5 and 12 September 2006 as well as 3 and 14 September 2007) with 'Pentakeep V' in a dose of 0.02% w/v (16 cm³ 100 dm⁻³). Solution was applied in the amount of 3000 dm³ per hectare according to the producer's recommendation (Cosmo Seiwa Agriculture Co., LTD. Japan). The following combinations with nitrogen soil fertilization were distinguished within each sub-block: 1 – control (unfertilized with nitrogen), 2 – 50% dose of

N prior to seed sowing (25 mg N dm⁻³ of soil), 3 – 100% dose of N prior to seed sowing (50 mg N dm⁻³ of soil). Pre-sowing nitrogen fertilization was carried out in the form of ammonium nitrate (Zakłady Azotowe in Puławy). 'Pentakeep V' fertilizer contains (in gravimetric percent): 9.5% N (3.8% N-NO₃, 5.7% N-NH₄), 5.7% MgO, 0.14% B, 0.02% Cu, 0.6% Fe-DTPA, 0.23% Mn, 0.02% Mo and 0.16% Zn and 5-aminolevulinic acid in concentration not declared by the producer. The experiment was carried out using a split-plot method in four replications (containers), each consisting of 4 rows with 10 plants per row. Seeds sowings were performed on 1th August in both years of the study using 15 seeds in one row. After germination plants were thinned out leaving 10 seedlings in one row (40 plants per one container). Spinach plants were harvested on 19th and 18th September in the subsequent years.

Each year, shredded plant material (spinach leaves) was dried at 70°C and mineralized in 65% super pure HNO₃ (Merck no. 100443.2500) in a CEM MARS-5 Xpress microwave oven [Paślowski and Migaszewski 2006]. In mineralized plant material concentration of Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V was determined using the ICP-OES technique with the use of a Prodigy Teledyne Leeman Labs USA spectrometer.

In both years of study, prior to the experiment, organic matter concentration in soil was determined using Tiurin method modified by Oleksynowa. Soil pH_(H₂O) was assessed by potentiometer, total concentration of salt in soil EC was measured conductometrically. The content of N-mineral (N-NH₄, N-NO₃), Ca, K, Mg and P was determined after extraction 0.03M CH₃COOH [Nowosielski 1988]. Nitrogen level was estimated by FIA technique [PN-EN ISO 13395:2001, PN-EN ISO 11732:2005 (U)], Ca, K and Mg were assessed by AAS method, while P with the use of vanadium-molybdenum method [Ostrowska et al. 1991]. Only in 2007, concentration of Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V after extraction 0.01 M CaCl₂ [Houba et al. 1997] in soil after the harvest were determined by ICP-OES method.

Obtained results were statistically verified by ANOVA module of Statistica 8.0 PL programme for significance level P < 0.05. Significance of changes was assessed with the use of variance analysis. In case of significant changes homogenous groups were determined on the basis of Duncan test.

RESULTS AND DISCUSSION

Meteorological data obtained throughout the research period was presented in table 1. During spinach cultivation year 2006 was characterized by lower values of average air temperature (measured daily) in August while higher in the first two decades of September in comparison to 2007. Total amount of rainfall in 2006 was 2.6 times lower than in the subsequent year but its distribution was more even. In the first decade of August 2007 a relatively small amount of rain and almost a double number of sunshine hours was noted in comparison to the respective period in 2006. On the other hand, first decade of September 2007 was characterized by 4.6 times lower number of sunshine hours and 18.8 times higher total amount of rainfall when compared to analogous time in the previous year. In 2006, during the first and the third decade of August higher air

humidity was noted in respect of 2007. In both years of spinach cultivation a comparable total number of sunshine hours was observed.

Table 1. Meteorological data throughout spinach cultivation in 2006 and 2007 (mean daily values)

Tabela 1. Dane meteorologiczne w okresie uprawy szpinaku w latach 2006 i 2007 roku (średnie dobowe wartości)

Year Rok	Month Miesiąc	Decade Dekada	Average air temperature Średnia tempera- tura powietrza °C	Rainfall Opady mm	Sunshine Usłonecznienie h	Humid air RH, % Wilgotność powietrza WW, %
2006	August – sierpień	I	18.8	35.1	36.4	83.4
		II	20.2	10.3	63.1	73.7
		III	16.4	58.7	55.5	82.1
	September – wrzesień	I	16.8	15.3	68.4	76.5
		II	16.8	1.0	64.5	76.1
	sum – suma		-	120.4	287.9	-
2007	August – sierpień	I	20.9	0.2	71.0	61.0
		II	20.4	10.4	55.1	78.2
		III	19.2	14.0	81.0	68.2
	September – wrzesień	I	13.1	288.0	14.6	86.7
		II	12.1	0.8	56.7	79.9
	sum – suma		-	313.4	278.4	-

Concentration of tested elements in spinach leaves and soil after spinach cultivation. The content of Ag, Al, Ba, Co, Ga, Sr Ti and V in spinach leaves depended on significant interaction between plant biostimulation with ‘Pentakeep V’ and nitrogen fertilization (table 2 – means for cooperation foliar nutrition × nitrogen fertilization). No significant cooperation of these factors was found in respect of the content of Cd, Cr, Li, Ni, Pb and Sb in spinach leaves. In control plants without N fertilization, foliar application of ‘Pentakeep V’ led to statistically significant reduction in the content of Ag, Al, Ba, Ga, Sr and Ti in spinach when compared to plants not treated with this substance. Among these six elements, only in the case of Sr a noticeable effect of ‘Pentakeep V’ was observed in plants fertilized with nitrogen. Foliar application of ‘Pentakeep V’ on plants fed with 100% dose of N resulted in greater accumulation of Sr in leaves in comparison to plants without foliar nutrition. Foliar nutrition with ‘Pentakeep V’ did not though influenced the content of strontium in plants nourished with 50% dose of nitrogen. Regarding Ag, Al, Ba, Ga and Ti accumulation in spinach leaves, ‘Pentakeep V’ application did not significantly affect its concentration in plants fertilized with both nitrogen doses (in comparison to plants without foliar nutrition). Results of cobalt concentration assessment revealed that the lowest level of this element was found in plants fertilized with 50% dose of N and not treated with ‘Pentakeep V’. Spinach plants from remaining combinations were characterized by significantly higher, comparable to each other, content of Co. In the case of vanadium, foliar nutrition of ‘Pentakeep V’ caused a slight tendency to decrease content of this element in control plants as well as improve V accumulation in plants fertilized with 50% dose of N.

Table 2. Content of Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V in spinach (means from 2006–2007)
 Tabela 2. Zawartość Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti i V w szpinaku (średnie z lat 2006–2007)

	Combinations – Kombinacje	mg·kg ⁻¹ d.w. – mg·kg ⁻¹ s.m.													
		Ag	Al	Ba	Cd	Co	Cr	Ga	Li	Ni	Pb	Sb	Sr	Ti	V
Means for cooperation	control – kontrola	0.40b	451.2b	12.5b	2.6a	0.34b	2.0a	5.5c	1.2a	1.5a	2.6a	0.80a	34.3d	67.8b	0.89b
foliar nutrition	50% dose of N	0.30a	242.4a	8.4a	2.9a	0.20a	1.5a	3.6a	0.8a	1.3a	2.6a	0.93a	27.3a	25.5a	0.46a
without foliar nutrition	50% dawki N														
nitrogen fertilization	100% dose of N	0.33a	308.1a	9.5a	3.4a	0.27b	1.8a	4.0ab	1.0a	1.6a	2.7a	1.03a	27.4a	31.4a	0.62a
without foliar nutrition	100% dawki N														
Średnie dla współpracy	control – kontrola	0.32a	335.1a	10.0a	2.6a	0.27b	1.8a	4.0ab	1.0a	1.4a	2.7a	0.65a	30.4c	33.1a	0.67ab
a dokarmiania	50% dose of N	0.30a	335.6a	9.4a	2.7a	0.29b	1.8a	4.1ab	1.0a	1.5a	3.3a	0.87a	27.8ab	32.4a	0.67ab
dolistnego × nawożenie	50% dawki N														
azotem	100% dose of N	0.33a	300.8a	9.4a	3.4a	0.29b	1.8a	4.6b	0.9a	1.5a	2.8a	0.87a	29.6bc	43.7a	0.58a
without foliar nutrition	100% dawki N														
Means for nitrogen fertilization	control – kontrola	0.36b	393.2b	11.3b	2.6a	0.30a	1.9a	4.8b	1.1a	1.5ab	2.7a	0.72a	32.3b	50.5b	0.78b
Średnie dla nawożenia	50% dose of N – 50% dawki N	0.30a	289.0a	8.9a	2.8a	0.25a	1.7a	3.9a	0.9a	1.4a	2.9a	0.90a	27.6a	28.9a	0.57a
azotem	100% dose of N – 100% dawki N	0.33a	304.4a	9.5a	3.4b	0.28a	1.8a	4.3ab	0.9a	1.6b	2.7a	0.95a	28.5a	37.5ab	0.60a
without foliar nutrition															
foliar nutrition	without foliar nutrition	0.34b	333.9a	10.1a	3.0a	0.27a	1.8a	4.4a	1.0a	1.5a	2.6a	0.92a	29.7a	41.5a	0.66a
dokarmiania	bez dokarmiania dolistnego														
Średnie dla dokarmiania	Pentakeep V	0.32a	323.8a	9.6a	2.9a	0.28a	1.8a	4.2a	1.0a	1.5a	2.9a	0.80a	29.3a	36.4a	0.64a
dolistnego															
Test <i>F</i> for cooperation × year of study	foliar nutrition × nitrogen fertilization	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Test <i>F</i> dla dokarmiania	dokarmiania dolistnego × nawożenie azotem × rok badań	*	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.

Means followed by the same letters are not significantly different for $P < 0.05$ – Średnie oznaczone tymi samymi literami nie różnią się istotnie dla $P < 0.05$
 Test *F*. * – means are significantly different, n.s. – not significant – średnie różnią się istotnie, n.i. – brak istotnego zróżnicowania

Table 3. Content of Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V in soil after spinach cultivation (results only for 2007)
 Tabela 3. Zawartość Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti i V w glebie po uprawie szpinaku (wyniki tylko dla 2007 roku)

	Combinations – Kombinacje	mg.kg ⁻¹ d.w. – mg.kg ⁻¹ s.m.													
		Ag	Al	Ba	Cd	Co	Cr	Ga	Li	Ni	Pb	Sb	Sr	Ti	V
Means for cooperation	control – kontrola	<0.004	707.4c	48.4c	0.649b	0.83a	0.47a	0.61a	0.157abc	2.23a	13.1a	<0.031	11.43d	0.346b	2.13a
foliar nutrition × bez dokarmiania dolist.	50% dose of N 50% dawki N	<0.004	669.2b	47.1b	0.640ab	0.77a	0.44a	0.59a	0.154ab	2.17a	13.5a	<0.031	10.76c	0.351bc	1.98a
nitrogen fertilization	100% dose of N 100% dawki N	<0.004	670.2b	47.2b	0.644b	0.82a	0.44a	0.59a	0.152a	2.28a	14.5a	<0.031	10.51a	0.330ab	1.93a
Średnie dla współdziałani	control – kontrola	<0.004	650.6a	45.7a	0.627a	0.96a	0.45a	0.62a	0.152a	2.21a	14.1a	<0.031	10.79c	0.322a	2.06a
a dokarmiania dolistnego × nawożenie azotem	50% dose of N 50% dawki N	<0.004	661.3ab	45.9a	0.650b	0.93a	0.45a	0.61a	0.161bc	2.28a	12.9a	<0.031	10.61ab	0.370cd	2.04a
	100% dose of N 100% dawki N	<0.004	669.2b	46.8ab	0.654b	0.88a	0.45a	0.61a	0.164c	2.32a	14.6a	<0.031	10.64b	0.380d	2.03a
Means for nitrogen fertilization	control – kontrola	<0.004	679.0b	47.1a	0.638a	0.89a	0.46a	0.62a	0.154a	2.22a	13.6a	<0.031	11.11c	0.334a	2.10a
Średnie dla nawożenia azotem	50% dose of N – 50% dawki N	<0.004	665.2a	46.5a	0.645a	0.85a	0.45a	0.60a	0.158a	2.23a	13.2a	<0.031	10.68b	0.360b	2.01a
	100% dose of N – 100% dawki N	<0.004	669.7ab	47.0a	0.649a	0.85a	0.44a	0.60a	0.158a	2.30b	14.5a	<0.031	10.58a	0.355b	1.98a
Means for foliar nutrition	without foliar nutrition bez dokarmiania dolistnego	<0.004	682.3b	47.6b	0.644a	0.81a	0.45a	0.60a	0.154a	2.22a	13.7a	<0.031	10.90b	0.342a	2.02a
Średnie dla dokarmiania dolistnego	Pentakeep V	<0.004	660.4a	46.1a	0.643a	0.92b	0.45a	0.61a	0.159b	2.27b	13.8a	<0.031	10.68a	0.357b	2.04a

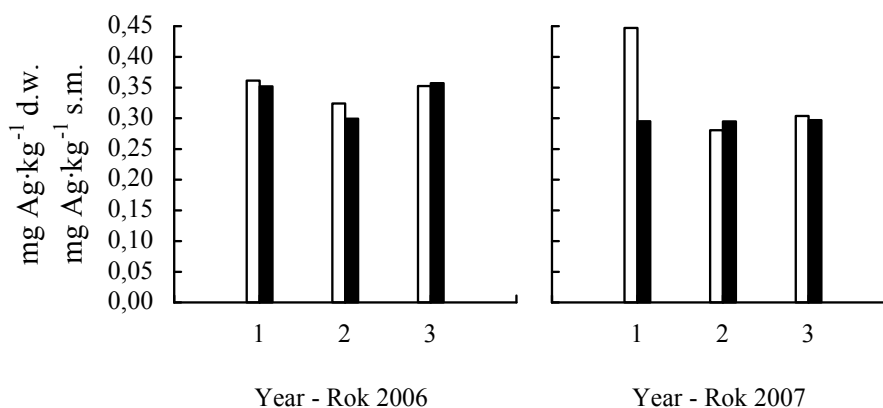
i – “<” means that concentrations of readily soluble forms of elements in soil were below the limit of its detection on the ICP-OES spectrometer. Means followed by the same letters are not significantly different for P < 0.05.

i – “<” oznacza, że zawartość łatwo rozpuszczalnych form pierwiastków w glebie była niższa od limitu ich detekcji na spektrometrze ICP-OES. Średnie oznaczone tymi samymi literami nie różnią się istotnie dla P < 0,05.

Diverse climatic conditions throughout both years of plant cultivation significantly affected interaction of foliar nutrition and nitrogen fertilization only in respect of Ag content in spinach leaves (Test *F* for cooperation foliar nutrition × nitrogen fertilization × year of study – table 2 and figure 1). That dependency was not observed for the content of Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V in spinach leaves – detailed data not presented. Only in 2007 'Pentakeep V' application contributed to reduction of Ag level in control spinach plants (not fertilized with N) in comparison to plants without foliar nutrition (fig. 1). In other combinations, foliar application of this substance had no significant effect on the leaf accumulation of Ag in both years of the study. Interpretation of obtained results on the basis of soil analysis causes difficulties as Ag concentration in soil in 2007 was below the limit of its detection using ICP-OES spectrometer.

LSD for foliar nutrition×nitrogen fertilization×year of study = 0.08

NIR dla dokarmiania dolistnego×nawożenie azotem×rok badań = 0,08



1 – Control – Kontrola, 2 – 50% dose of N – 50% dawki N, 3 – 100% dose of N – 100% dawki N
 □ – sub-block without foliar nutrition – podblok bez dokarmiania dolistnego, ■ – sub-block with foliar application of 'Pentakeep V' – podblok z dolistną aplikacją Pentakeep V

Fig. 1. Content of Ag in spinach in relation to foliar application of 'Pentakeep V' and nitrogen fertilization in 2006–2007

Ryc. 1. Zawartość Ag w szpinaku w latach 2006-2007 w zależności od dolistnej aplikacji Pentakeep V i dogłębowego nawożenia azotem.

Observed significant differences in cooperation between foliar nutrition and nitrogen fertilization on the content of: Ag, Al, Ba, Co, Ga, Sr Ti and V in spinach leaves had no relation with noted diversity in the content of these elements in soil after spinach harvest (tables 2 and 3 – means for cooperation foliar nutrition × nitrogen fertilization). No correlation was found between changes in soil content of Al, Ba, Cd, Li, Sr and Ti in combinations with nitrogen fertilization in both sub-blocks and its concentration in plant

material. Only in respect of Al, Ba Sr and Ti, reduction of its content in control plants with ‘Pentakeep V’ application (in comparison to control plants without foliar nutrition) could have been caused by lower concentration of these elements in soil. Additionally, increased concentration of Sr in soil fertilized with 100% dose of N in combination with foliar application of ‘Pentakeep V’ (in relation to the combination in sub-block without foliar nutrition) might have contributed to greater accumulation of strontium in spinach leaves. It should be also added that the soil content of Co, Cr, Ga, Ni, Pb and V was comparable for both sub-blocks. As it was mentioned before, the concentration of Ag and Sb forms soluble in 0.01 M CaCl₂ was below the limits of its detection by ICP-OES spectrometer.

Foliar application of ‘Pentakeep V’ (regarded irrespective of nitrogen fertilization, table 1 – means for foliar nutrition) led to decreased content of Ag and did not affect the level of Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V in spinach leaves. Relatively comparable content of these elements in plants from both sub-blocks was noted along with highly diversified concentration of Al, Ba, Co, Li, Ni, Sr and Ti in soil (table 2 – means for foliar nutrition).

Only few studies documented how ALA influenced mineral balance in plants and mainly presented the effect of ALA or Pentakeep® fertilizers on macro-element nutrition of plants [Awad 2008; Babik and Babik 2007, 2008; Babik et al. 2008; Ježek and Kopecký 2008; Nowak 2007]. The impact of ALA on nitrate(V) metabolism in plants was also discussed [Tanaka et al. 2005] as well as improvement of salt tolerance in cotton seedlings through the reduction in sodium uptake [Watanabe et al. 2000].

Burzynski [1985] has shown that lead inhibited delta aminolevulinic acid dehydratase activity (ALA-D - an important enzyme of chlorophyll biosynthesis). Results by Pereira et al. [2006] demonstrated that aluminum inhibits enzyme ALA-D and also greatly impairs plant growth. ALA-D inhibition may occur due to the fact that aluminum present in the growth medium can compete with Mg²⁺ or reduce the expression of ALA-D. Still, no information is available on how application of exogenous ALA could affect uptake and accumulation of microelements, heavy metals or trace elements by plants. Thus, objective discussion referring to other authors’ works remains a challenging task. What causes additional difficulties is the fact that results shown in the present study refer to interaction of foliar nutrition and soil fertilization with nitrogen on the uptake and level of fourteen elements in plants. Each of them can be described by diverse chemical characteristics, mobility in soil – plant system as well as different mechanisms of uptake and impact on plants [Kabata-Pendias and Mukherjee 2007, Tyler and Olsson 2001].

The influence of mineral nitrogen fertilization on the level of phytoavailable forms of heavy metals in soil (and its accumulation in plants as a consequence) depends on type of nitrogen fertilizer, its dose as well as time of application. Nitrogen fertilization can lead to lower accumulation of heavy metals in plants due to dilution effect resulting from intensified plant growth. Application of physiologically acid nitrogen fertilizers can increase soil concentration of heavy metal forms available for plants. It then can contribute to greater accumulation of these elements in plants [Gębski 1998]. Jurkowska and Rogóż [1981] showed that along with increasing dose of ammonium nitrate applied in soil higher level of Mn, Zn, Cu and B, while lower of Fe and Mo, were noted in sor-

rel. In the research conducted by Smoleń and Sady [2009] nitrogen fertilization (in the form of calcium nitrate, ammonium nitrate, ammonium sulphate and urea) resulted in higher concentration of Mg and Se as well as caused no significant changes in the content of K, Ca, Na, Al, B, Bi, Fe, Ga, Ti, As, Co, Cr, In, Li, Ni, Pb, Sb and V in carrot storage roots when compared to plants not fertilized with N. These authors also revealed that accumulation of Ba, Mn, Sr, Be and Mo in carrot roots depended on the form of N fertilizer. In the present study, the analysis of concentration of tested elements in relation to N fertilization (considered irrespective of foliar nutrition, table 2 – means for fertilization) showed that nitrogen application had a significant influence on the content of Ag, Al, Ba, Cd, Ga, Ni, Sr, Ti and V in spinach leaves. No significant effect was observed in plant concentration of Co, Cr, Li, Pb and Sb as a relation to diverse doses of N fertilization. Both doses of N fertilization, in comparison to control, caused a significant reduction in the content of Ag, Al, Ba, Sr, and V in spinach leaves. In the case of Ga and Ti its content was the lowest in plants fertilized with 50% dose of N. The highest concentration of Cd was observed in plants fertilized with 100% dose of N. The lowest accumulation of nickel, but comparable to control, was determined in plants fertilized with 50% dose of N. It should be noted that reduction in the level of Al and Sr in spinach plants fertilized with both nitrogen doses (tab. 2) could have been related with a decrease in the content of available forms of these elements in soil (tab. 2). Observed changes in the accumulation of nickel in spinach were also supported by results of its determination in soil. In the case of Ba, Cd, Co, Cr, Ga, Li, Pb and V nitrogen fertilization had no significant influence on its accumulation in soil.

CONCLUSIONS

In comparison to plants without foliar nutrition, application of 'Pentakleep V' resulted in: a) significant reduction in the content of: Ag, Al, Ba, Ga, Sr and Ti in control plants not fertilized with nitrogen, b) increase in the accumulation of Sr in spinach plants fertilized with 100% dose of N, c) relatively weak tendency to decrease V concentration in control plants.

The lowest accumulation of cobalt was observed in plants fertilized with 50% of N dose without foliar application of 'Pentakleep V'.

Obtained significant differences in interaction between foliar nutrition and nitrogen fertilization in respect of Ag, Al, Ba, Co, Ga, Sr, Ti and V concentration in spinach had no relation to noted diverse level of these elements in soil.

No interaction between foliar nutrition and nitrogen fertilization was found in reference to Cd, Cr, Li, Ni, Pb and Sb content in spinach plants.

A significant effect of climatic conditions throughout spinach cultivation on diversified interaction of foliar nutrition and nitrogen fertilization was observed only in 2007 in respect of Ag content in control plants.

Both does of nitrogen fertilization (regarded independently of foliar nutrition) caused a significant reduction of Ag, Al, Ba, Ga, Sr, Ti and V accumulation in spinach when compared to control plants.

Nitrogen fertilization in 100% dose of N contributed to higher level of Cd while 50% dose of N resulted in decrease of Ni concentration in spinach plants.

Foliar application of 'Pentakeep V' (irrespective of nitrogen fertilization) led to reduction in Ag accumulation and had no significant effect on Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti and V content in spinach plants.

REFERENCES

- Awad M.A., 2008. Promotive effects of a 5-aminolevulinic acid-based fertilizer on growth of tissue culture-derived date palm plants (*Phoenix dactylifera* L.) during acclimatization. *Sci. Hort.* 118 (1), 48–52.
- Babik I., Babik J., 2007. Effect of Pentakeep-V on the yield and quality of leek. *Proceedings. Pentakeep International Scientific Workshop 2006 in Budapest.* Budapest, Hungary, December 10–11, 2006. Cosmo Oil Co., LTD. Japan, 250–253.
- Babik I., Babik J., 2008. Pentakeep® in vegetable production – filed crops: tomato and white cabbage. *Proceedings. Pentakeep International Scientific Workshop 2007 in Prague.* Prague, Czech Republic, December 7–9, 2007. Cosmo Oil Co., LTD. Japan, 212–225.
- Babik I., Babik J., Dyśko J., 2008. Effect of 5-aminolevulinic acid (ALA) from Pentakeep® fertilizers on yield and quality of vegetables grown in the field and under covers. [In:] *Biostimulators In Modern Agriculture. Vegetable Crops. Monograph* (Z. T. Dąbrowski ed.). Publisher Editorial House Wieś Jutra, Warszawa, 61–74.
- Burzynski M., 1985. Influence of lead on the chlorophyll content and on initial steps on its synthesis in greening cucumber seedlings. *Acta Soc. Bot. Poll.* 54, 95–105.
- Gębski M., 1998. Czynniki glebowe oraz nawozowe wpływające na przyswajanie metali ciężkich przez rośliny. *Post. Nauk Rol.* 5, 3–16.
- Houba V.J.G., Novozamsky I., Temminghoff E., 1997. Soil analysis procedures. Extraction with 0.01 M CaCl₂ (Soil and Plant Analysis, Part 5A). Wageningen Agricultural University, The Netherlands.
- Ježek J., Kopecký J., 2008. Effect of EC fertilizer Pentakeep Super on the yield and on content of alpha-acid in hops. *Proceedings. Pentakeep International Scientific Workshop 2007 in Prague.* Prague, Czech Republic, December 7–9, 2007. Cosmo Oil Co., LTD. Japan, 62–90.
- Jurkowska H., Rogóż A., 1981. Wpływ formy i dawki azotu na zawartość makro- i mikroelementów w roślinach. Cz. II: Mikroelementy. *Acta Agr. Silv., Ser. Agr.* 20, 121–131.
- Kabata-Pendias A., Mukherjee A.B., 2007. Trace elements from soil to human. Springer-Verlag Berlin Heidelberg.
- Memon S.A., Hou X., Wang L., Li Y., 2009. Promotive effect of 5-aminolevulinic acid on chlorophyll, antioxidative enzymes and photosynthesis of Pakchoi (*Brassica campestris* ssp. *chinensis* var. *communis* Tsen et Lee). *Acta Physiol. Plant.* 31, 51–57.
- Nowak J., 2007. Effect of 5-aminolevulinic acid and mineral nutrition (Pentakeep V) on growth and flowering of chrysanthemum. *Proceedings. Pentakeep International Scientific Workshop 2006 in Budapest.* Budapest, Hungary, December 10–11, 2006. Cosmo Oil Co., LTD. Japan, 198–210.
- Nowosielski, O., 1988. *Zasady opracowywania zaleceń nawozowych w ogrodnictwie.* PWRiL, Warszawa.
- Ostrowska A., Gawaliński S., Szczubiałkowska Z., 1991. *Metody analiz i oceny właściwości gleb i podłoży – katalog.* Instytut Ochrony Środowiska, Warszawa.

- Pasławski P., Migaszewski Z.M., 2006. The quality of element determinations in plant materials by instrumental methods. Polish J. Environ. Stud. 15(2a), Part I, 154–164.
- Pereira L.B., Tabaldi L.A., Gonçalves J.F., Jucoski G.O., Pauletto M.M., Weis S.N., Nicoloso F., T., Borher D., Rocha J.B.T., Schetinger M.R.C., 2006. Effect of aluminum on aminolevulinic acid dehydratase (ALA-D) and the development of cucumber (*Cucumis sativus*). Environ. Exp. Bot. 57, 106–115.
- PN-EN ISO 11732:2005 (U). Jakość wody. Oznaczanie azotu amonowego metodą analizy przepływowej (CFA i FIA) z detekcją spektrometryczną.
- PN-EN ISO 13395:2001. Jakość wody. Oznaczanie azotu azotynowego i azotanowego oraz ich sumy metodą analizy przepływowej (CFA i FIA) z detekcją spektrofotometryczną.
- Smoleń S., 2008. Wpływ dokarmiania dolistnego azotem, molibdenem, sacharozą i benzyloadniną na zawartość Cd, Fe, Mn, Pb i Zn w rzodkiewce. Annales UMCS, sec. E, Agricultura 63 (4), 34–41.
- Smoleń S., Sady W., 2008. The effect of foliar nutrition with nitrogen, molybdenum, sucrose and benzyladenine on the contents of dry weight, Cd, Cu and Zn in carrot. Veget. Crops Res. Bull. 68, 135–144.
- Smoleń S., Sady W., 2009. The effect of nitrogen fertilizer form and foliar application on concentrations of twenty five elements in carrot. Folia Hort. 21 (1), 3–16.
- Tanaka T., Iwai K., Watanabe K., Hotta Y., 2005. Development of 5-aminolevulinic acid for agriculture uses. Regul. Plant Growth Devel., 40 (1), 22–29.
- Tyler G., Olsson T., 2001. Concentrations of 60 elements in the soil solution as related to the soil acidity. Europ. J. Soil Sci. 52, 151–165.
- Ustawa o nawozach i nawożeniu z dnia 10 lipca 2007 r. Dz. U. 2007 nr 147, poz. 1033.
- Watanabe, K., Tanaka, T., Hotta, Y., Kuramochi, H., Takeuchi, Y., 2000. Improving salt tolerance of cotton seedlings with 5-aminolevulinic acid. J. Plant Growth Regul. 32, 99–103.
- Wierzbńska J., 2009. Oddziaływanie zabiegu dokarmiania dolistnego składnikami odżywczymi w połączeniu z fitohormonami i kwasami organicznymi na wielkość i jakość biologiczną plonu marchwi (*Daucus carota* L.). Praca magisterska. Katedra Uprawy Roli i Nawożenia Roślin Ogrodniczych, Wydział Ogrodniczy, Uniwersytet Rolniczy w Krakowie.
- Yaronskaya E., Vershilovskaya I., Poers Y., Alawady A.E., Averina N., Grimm B., 2006. Cytokinin effects on tetrapyrrole biosynthesis and photosynthetic activity in barley seedlings. Planta 224, 700–709.

WPLYW BIOSTYMULACJI ROŚLIN NAWOZEM 'PENTAKEEP V' ORAZ NAWOŻENIA AZOTEM NA ZAWARTOŚĆ CZTERNASTU PIERWIASTKÓW W SZPINAKU

Streszczenie. Dolistna aplikacja regulatorów wzrostu lub nawozów zawierających biostymulatory mogą wpływać na pobieranie i akumulację składników mineralnych przez rośliny. Celem badań było określenie wpływu dokarmiania dolistnego nawozem 'Pentakeep V' oraz zróżnicowanego pod względem dawki nawożenia azotem na zawartość: Ag, Al, Ba, Cd, Co, Cr, Ga, Li, Ni, Pb, Sb, Sr, Ti i V w szpinaku. W latach 2006–2007 przeprowadzono doświadczenie wazonowe z uprawą szpinaku *Spinacia oleracea* L. 'Spinaker F₁'. Szpinak uprawiano w glinie średniej pylastej. Badaniami objęto dwa podbloki z dolistnym i bez dolistnego dokarmiania roślin. Rośliny dokarmiano dolistnie dwukrotnie nawozem 'Pentakeep V' w dawce 0,02% m/o, stosując w przeliczeniu 3000 dm³ wody na

hektar. W obrębie podbloków zastosowano dogłębne przedsiewne nawożenie azotem (w formie saletry amonowej): 1 – kontrola (nienawożona azotem), 2 – 50% dawki N ($25 \text{ mg N} \cdot \text{dm}^{-3}$ gleby), 3 – 100% dawki N ($50 \text{ mg N} \cdot \text{dm}^{-3}$ gleby). Dolistna aplikacja ‘Pentakeep V’ w porównaniu do roślin niedokarmianych dolistnie powodowała: a) istotne zmniejszenie zawartości Ag, Al, Ba, Ga, Sr i Ti w roślinach kontrolnych nienawożonych azotem, b) zwiększenie zawartości Sr w szpinaku nawożonym 100% dawki N, c) stosunkowo niewielką tendencję do obniżenia zawartości wanadu w roślinach kontrolnych. Najniższą zawartością kobaltu charakteryzowały się rośliny nawożone 50% dawki N i niedokarmiane dolistnie. Nie stwierdzono istotnego wpływu współdziałania dokarmiania dolistnego Pentakeep V i nawożenia azotem na zawartość Cd, Cr, Li, Ni, Pb i Sb w szpinaku.

Słowa kluczowe: dokarmianie dolistne, skład mineralny, metale ciężkie, pierwiastki śladowe

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