

# PRELIMINARY EVALUATION OF THE INFLUENCE OF IODINE AND NITROGEN FERTILIZATION ON THE EFFECTIVENESS OF IODINE BIOFORTIFICATION AND MINERAL COMPOSITION OF CARROT STORAGE ROOTS

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## Abstract

Biofortification of vegetables with iodine can become an alternative method to salt iodization of introducing this element to human diet. Iodine is not an essential nutrient for plants and its effect on plant growth and development has not yet been sufficiently examined. The aim of the study was to assess the influence of soil fertilization with iodine (in the form of  $I^-$  and  $IO_3^-$ ) and nitrogen (applied as  $NO_3^-$  and  $NH_4^+$ ) on the effectiveness of iodine biofortification as well as mineral composition of carrot storage roots. Carrot cv. Kazan F<sub>1</sub> was cultivated in a field experiment in 2008 and 2009. Different soil fertilization treatments with iodine as well as nitrogen were tested, including: 1 – control without N and I fertilization; 2 – KI fertilization without N application; 3 –  $KIO_3$  fertilization without N application; 4 – KI +  $Ca(NO_3)_2$  fertilization; 5 –  $KIO_3$  +  $Ca(NO_3)_2$  fertilization, 6 – KI +  $(NH_4)_2SO_4$  fertilization, 7 –  $KIO_3$  +  $(NH_4)_2SO_4$  fertilization. Iodine as KI and  $KIO_3$  was applied pre-sowing in a dose of 2 kg I ha<sup>-1</sup>. Nitrogen fertilization in the form of  $Ca(NO_3)_2$  and  $(NH_4)_2SO_4$  was performed pre-sowing and as top dressing with 100 kg N ha<sup>-1</sup>. In carrot storage roots, the iodine content as well as P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb concentration were determined by the ICP-OES technique, while nitrogen – using Kjeldahl method. Better results of iodine enrichment in carrot were obtained

after introducing this element in the form of KI, especially together with ammonium sulphate. Application of the  $\text{IO}_3^-$  form of iodine significantly improved nitrogen utilization from mineral fertilizers by carrot plants. In storage roots of carrots cultivated without N nutrition, iodine treatment (in both forms: KI and  $\text{KIO}_3$ ) contributed to a significant increase in P, K and Ca content as well as a reduction in Fe accumulation. However, it had no influence on the concentration of Mg, S, Cu, Mn, Zn, Mo, Al and Pb in carrot storage roots. Application of  $\text{KIO}_3$ , in comparison to KI, resulted in a significant increase of the K, Fe and Zn content in carrot roots fertilized with  $\text{Ca}(\text{NO}_3)_2$ . In the case of  $(\text{NH}_4)_2\text{SO}_4$  as a nitrogen source,  $\text{KIO}_3$  contributed to significantly higher accumulation of P, K, Mg, S, Na, B, Cu, Fe, Mn, Al and Cd in carrot storage roots when compared to KI.

Key words: biofortification, iodine, nitrogen fertilization, mineral composition, carrot.

## WSTĘPNA OCENA WPŁYWU NAWOŻENIA JODEM I AZOTEM NA EFEKTYWNOŚĆ BIOFORTYFIKACJI MARCHWI W JOD ORAZ NA JEJ SKŁAD MINERALNY

### Abstrakt

Warzywa biofortyfikowane jodem mogą być alternatywnym, do jodowania soli, sposobem wprowadzenia tego pierwiastka do diety człowieka. Jod nie jest pierwiastkiem niezbędnym dla roślin. Jego oddziaływanie na rośliny nie zostało dostatecznie zdiagnozowane. Celem badań było określenie wpływu doglebowego nawożenia jodem (w formie  $\text{I}^-$  i  $\text{IO}_3^-$ ) i azotem (w formie  $\text{NO}_3^-$  i  $\text{NH}_4^+$ ) na efektywność biofortyfikacji jodem oraz na skład mineralny marchwi. Marchew odmiany Kazan  $\text{F}_1$  uprawiano w doświadczeniu polowym w latach 2008-2009. W badaniach zastosowano zróżnicowane doglebowe nawożenie jodem (I w formie  $\text{I}^-$  lub  $\text{IO}_3^-$ ) i azotem (N w formie  $\text{NO}_3^-$  lub  $\text{NH}_4^+$ ): 1 – kontrola bez nawożenia N i I, 2 – nawożenie KI bez nawożenia N, 3 – nawożenie  $\text{KIO}_3$  bez nawożenia N, 4 – nawożenie KI +  $\text{Ca}(\text{NO}_3)_2$ , 5 – nawożenie  $\text{KIO}_3$  +  $\text{Ca}(\text{NO}_3)_2$ , 6 – nawożenie KI +  $(\text{NH}_4)_2\text{SO}_4$ , 7 – nawożenie  $\text{KIO}_3$  +  $(\text{NH}_4)_2\text{SO}_4$ . Jod w formie KI i  $\text{KIO}_3$  aplikowano przedsięwzięcie w dawce  $2 \text{ kg I ha}^{-1}$ , azot w formie  $\text{Ca}(\text{NO}_3)_2$  i  $(\text{NH}_4)_2\text{SO}_4$  – w dawce po  $100 \text{ kg N ha}^{-1}$  przedsięwzięcie i pogłównie. W marchwi oznaczono: zawartość jodu oraz P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd i Pb techniką ICP-OES; zawartość azotu metodą Kiejdahla. Lepsze efekty wzbogacania marchwi w jod uzyskano po zastosowaniu jodu w formie KI, zwłaszcza gdy stosowano ten związek w połączeniu z siarczanem amonu. Zastosowanie jodu w formie  $\text{IO}_3^-$  istotnie poprawiało efektywność wykorzystania azotu przez rośliny z zastosowanych nawozów mineralnych. W korzeniach spichrzowych roślin nienawożonych azotem zastosowanie jodu (w formie KI i  $\text{KIO}_3$ ) powodowało istotne zwiększenie zawartości P, K, Ca oraz zmniejszenie zawartości Fe; natomiast nie miało wpływu na zawartość Mg, S, Cu, Mn, Zn, Mo, Al i Pb. Zastosowanie  $\text{KIO}_3$ , w porównaniu z KI, powodowało istotne zwiększenie zawartości K, Fe i Zn w marchwi nawożonej  $\text{Ca}(\text{NO}_3)_2$ . W przypadku nawożenia  $(\text{NH}_4)_2\text{SO}_4$  aplikacja  $\text{KIO}_3$ , w porównaniu z KI, powodowała istotne zwiększenie zawartości P, K, Mg, S, Na, B, Cu, Fe, Mn, Al i Cd w marchwi.

Słowa kluczowe: biofortyfikacja, jod, nawożenie azotem, skład mineralny, marchew.

## INTRODUCTION

In Poland and many other countries around the world, iodine content in human diet is currently raised through salt iodization. Excessive salt consumption, however, has recently led to increased incidences of cardiovascu-

lar diseases. For that reason, the Global Strategy on Diet, Physical Activity and Health for the years 2008-2013 has been proposed by WHO. This program includes significant reduction of salt intake and recommends searching for alternative ways of introducing iodine to human diet. The need for global development of effective methods to enrich our diet in this element results from numerous functions that iodine plays in a human organism. Moreover, 35.2% of the global population suffers from an inadequate iodine nutrition (WINGER et al. 2008).

Iodine biofortification of vegetables can be perceived as one of the alternative ways of introducing iodine into human diet. Biofortification is defined as such a process that increases the content of biogenic elements such as Fe, Zn, Ca, Mg, Cu, I or Se in edible parts of crop plants. As a result, the consumer's health is expected to improve. Increased accumulation of biogenic elements in plants can be achieved through application of agronomic, genetic or transgenic strategies (STRZETELSKI 2005, WHITE, BROADLEY 2005, 2009, YANG et al. 2007, ZHAO, McGRATH 2009).

Iodine is not an essential nutrient for plant growth and development and its influence on plants has not yet been diagnosed (KABATA, MUKHERJEE 2007). In general, the research on iodine neglects problems in biofortification due to the interaction of iodine with physiological and biochemical processes occurring in plants, including mineral nutrition. Evaluation of these interrelations is crucial for developing optimal agrotechniques of plant biofortification with iodine.

At present, there are no norms for an acceptable level of iodine in vegetables. In the future, results of studies on iodine biofortification can help develop these standards in order to adequately balance the content of this element in human diet.

The aim of the study has been to determine the influence of soil fertilization with iodine (in the form of  $I^-$  and  $IO_3^-$ ) and nitrogen (as  $NO_3^-$  and  $NH_4^+$ ) on iodine biofortification efficiency and mineral composition of carrot storage roots.

## MATERIAL AND METHODS

In 2008-2009, a field experiment was conducted in Kraków (Poland), on carrot cv. Kazan F<sub>1</sub> grown in crop rotation on uniform soil complex. The carrot was cultivated on silt loam soil (35% sand, 28% silt and 37% clay) with the content of organic matter 2.84%-3.41% in the 0-30 cm soil layer and the following concentrations of the available forms of nutrients soluble in 0.03 M acetic acid: N ( $NO_3^-$ -N+ $NH_4^+$ -N) – 8.1-3.8 mg, P – 51.4-45.0 mg, K – 111.8-185.4 mg, Mg – 115.6-107.4 mg and Ca – 1255.8-837.9 mg in 1 dm<sup>-3</sup> of soil (in 2008 and 2009, respectively). In the subsequent years, the soil

pH<sub>(H<sub>2</sub>O)</sub> was 6.98-7.10 while the general concentration of salt in soil (EC) was 0.12-0.11 mS cm<sup>-1</sup>. The carrot was grown on ridges, 40 cm wide and 30 cm high, where seeds were sown in one row at a rate of 37 seeds m<sup>-1</sup> (approximately 550 000 seeds per 1 hectare). Seed sowing was performed on 24 April in both years of the study.

Several variants of soil fertilization with iodine (in the form of I<sup>-</sup> or IO<sub>3</sub>) and nitrogen (as NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup>) were applied in the experiment: 1 – control without N and I fertilization; 2 – KI application without N fertilization; 3 – KIO<sub>3</sub> application without N fertilization; 4 – KI + Ca(NO<sub>3</sub>)<sub>2</sub> fertilization; 5 – KIO<sub>3</sub> + Ca(NO<sub>3</sub>)<sub>2</sub> fertilization; 6 – KI + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> fertilization; 7 – KIO<sub>3</sub> + (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> fertilization. Iodine (in both forms) was applied pre-sowing in a dose of 2 kg I ha<sup>-1</sup>. The iodine dose used in the experiment was chosen on the basis of our previous studies (SMOLEŃ et al. 2009b, STRZETELSKI et al. 2010) as well as the results obtained by other authors (BORST PAUWELS 1961, SMITH, MIDDLETON 1982, ALTMOK et al. 2003).

Nitrogen in the forms of Ca(NO<sub>3</sub>)<sub>2</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was introduced pre-sowing and as top dressing, each dose of 100 kg N ha<sup>-1</sup>. The pre-sowing nitrogen fertilization (and iodine application) was conducted immediately before the ridge formation, whereas the top dressing – at canopy closure. The experiment was arranged in a split-plot design. Each experimental treatment was randomized in four replications on 2.7 m × 5 m (13.5 m<sup>2</sup>) plots. The total area used for the experiment was 378 m<sup>2</sup>.

The carrot was harvested on 30 and 23 September (in 2008 and 2009, respectively). During harvest, about 5 kg of carrot storage roots were taken in four replications (from each plot) for further analyses alongside soil samples from three layers (0-30 cm, 30-60 cm and 60-90 cm), which were collected using a soil drill.

In carrot storage roots, iodine content was assessed after sample incubation with 25% TMAH according to the standard project prEN 15111-R2-P5-F01, while N-total was determined using Kjeldahl method (PERSSON, WENNERHOLM 1999). The concentration of P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb was determined after mineralization in 65% super pure HNO<sub>3</sub> (Merck no 100443.2500) in a CEM MARS-5 Xpress microwave oven (PASŁAWSKI, MIGASZEWSKI 2006).

In soil samples, pH was determined with a potentiometer and concentrations of I, N-NH<sub>4</sub>, N-NO<sub>3</sub>, P, K, Mg, Ca, S and Na were determined after extraction with 0.03 M acetic acid (NOWOSIELSKI 1988). The content of B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb was assayed after soil extraction with 1 M HCl (GORLACH et al. 1999).

Iodine as well as P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Mo, Al, Cd and Pb in carrot and soil samples were determined with the ICP-OES technique using a Prodigy Teledyne Leeman Labs USA spectrometer. The concentration of nitrogen forms in soil samples (N-NH<sub>4</sub>, N-NO<sub>3</sub>) was deter-

mined with the FIA technique [PN-EN ISO 13395: 2001; PN-EN ISO 11732:2005 (U)].

The results were statistically verified using the ANOVA module of Statistica 9.0 PL programme at the significance level  $P < 0.05$ . Significance of changes was assessed with the use of variance analysis. For significant changes, homogenous groups were distinguished on the basis of Duncan test.

## RESULTS AND DISCUSSION

In comparison to the control, significantly increased iodine content in carrot storage roots was observed in all combinations with iodine fertilization in the form of KI (Table 1). The highest amount of this element was detected in roots of plants fertilized with KI and  $(\text{NH}_4)_2\text{SO}_4$  (combination 6). A slightly lower but still comparable level of iodine in carrot roots was found after fertilization with KI only (combination no.2) as well as KI together with calcium nitrate (combination no.4). It was revealed that soil application of  $\text{KIO}_3$  (along with nitrogen fertilizers) resulted in just a slight build-up of iodine in carrot storage roots. In storage roots of plants treated with  $\text{KIO}_3$ , a tendency for increasing iodine concentration (in comparison to the control) was observed but no statistical significance was noted. It should be mentioned that in all the tested combinations, iodine content in the 0-90 cm soil layer remained at a comparable level (Table 2). In our previous studies with pot cultivation of carrot (SMOLEŇ et al. 2009a) conducted in an analogous experiment design with iodine and nitrogen fertilization (on soil characterized by similar physicochemical properties), iodine applied as KI and  $\text{KIO}_3$  did not contribute to biofortification of carrot storage roots and leaves with this element. Exceptionally, increased concentration of iodine was found only in leaves of carrot treated with  $\text{KIO}_3$  (without N) as well as  $\text{Ca}(\text{NO}_3)_2 + \text{KI}$ . It should be underlined, however, that in that pot experiment iodine concentration in carrot was assayed after incubation of root and leaf samples in 2% acetic acid, not 25% solution of TMAH as in the present work. Thus, the analytical procedure applied for iodine determination (apart from different cultivation conditions) could be responsible for obtaining different results in reference to the effect of I and N fertilization on iodine biofortification of carrot.

Iodine application regarded together with differential nitrogen fertilization significantly affected the content of: N, P, K, Mg, Ca, S, Na, B, Cu, Fe, Mn, Zn, Al, Cd and Pb in carrot (Table 1). However, the iodine and nitrogen treatments tested in the experiment did not influence Mo concentration in carrot storage roots.

In the aforementioned pot cultivation of carrot (SMOLEŇ 2009, SMOLEŇ et al. 2009a), application of iodine in the form of  $\text{KIO}_3$  (in reference to KI),

Table 1

Mineral composition and the effectiveness of iodine biofortification of carrot storage roots depending on nitrogen and iodine fertilization – means from 2008-2009

Combinations	Content of iodine (mg I kg <sup>-1</sup> d.w.)	Content of macroelements in % d.w. of carrot storage roots									
		N	P	K	Mg	Ca	S	Na			
1. Control (without N and I)	2.0 a	1.42 a	0.350 ab	2.30 b	0.113 a	0.318 ab	0.13 a	0.76 d			
2. KI (without N)	4.3 cd	1.46 b	0.380 c	2.46 d	0.111 a	0.345 bc	0.13 a	0.76 d			
3. KIO <sub>3</sub> (without N)	2.6 ab	1.40 a	0.364 bc	2.38 c	0.112 a	0.348 c	0.13 a	0.81 e			
4. Ca(NO <sub>3</sub> ) <sub>2</sub> +KI	3.8 bcd	1.77 c	0.367 bc	2.45 d	0.128 b	0.337 abc	0.13 a	0.73 c			
5. Ca(NO <sub>3</sub> ) <sub>2</sub> +KIO <sub>3</sub>	3.4 abc	2.00 d	0.377 bc	2.50 e	0.132 bc	0.351 c	0.14 a	0.72 c			
6. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +KI	5.0 d	1.75 c	0.327 a	2.23 a	0.116 a	0.313 a	0.14 a	0.69 a			
7. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +KIO <sub>3</sub>	3.1 abc	1.99 d	0.381 c	2.55 f	0.135 c	0.339 abc	0.15 b	0.70 b			
Test F	*	*	*	*	*	*	*	*			
Content of microelements, Al, Cd and Pb in mg kg <sup>-1</sup> d.w. of carrot storage roots											
	B	Cu	Fe	Mn	Zn	Mo**	Al	Cd	Pb		
1. Control (without N and I)	24.5 c	4.8 a	47.2 c	6.3 ab	24.3 a	0.315	44.0 c	1.15 a	0.273 c		
2. KI (without N)	23.9 b	4.9 ab	39.5 ab	6.0 a	25.9 a	0.373	40.7 bc	1.27 b	0.226 bc		
3. KIO <sub>3</sub> (without N)	24.2 bc	4.6 a	40.8 ab	6.3 ab	24.4 a	0.325	43.8 c	1.16 a	0.161 abc		
4. Ca(NO <sub>3</sub> ) <sub>2</sub> +KI	24.2 bc	5.3 bc	40.1 ab	6.4 bc	29.9 b	0.360	28.0 b	1.25 ab	0.230 bc		
5. Ca(NO <sub>3</sub> ) <sub>2</sub> +KIO <sub>3</sub>	24.1 bc	5.6 c	41.8 c	6.6 c	33.2 c	0.385	39.8 bc	1.34 b	0.230 bc		
6. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +KI	23.0 a	5.2 b	33.5 a	11.5 d	30.9 bc	0.282	27.4 a	1.50 c	0.086 a		
7. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +KIO <sub>3</sub>	25.1 d	6.1 d	42.5 c	16.2 e	37.7 d	0.321	38.3 b	1.68 d	0.106 ab		
Test F	*	*	*	*	*	n.s.	*	*	*		

Means followed by the same letters are not significantly different for  $P < 0.05$ .

\* means are significantly different, n.s. – not significant, \*\* results for Mo from 2009 only

Table 2

Results of chemical analysis of soil after carrot cultivation – means for the 0-90 cm soil layer from 2008-2009

Combinations	pH <sub>H<sub>2</sub>O</sub>	(mg dm <sup>-3</sup> soil)									
		I	N-NH <sub>4</sub>	N-NO <sub>3</sub>	P	K	Mg	Ca	S	Na	
1. Control (without N and I)	7.23 <i>de</i>	1.15	0.3 <i>a</i>	1.3 <i>a</i>	16.2	23.2 <i>a</i>	75.3	998.5 <i>a</i>	7.8 <i>a</i>	4.5 <i>a</i>	
2. KI (without N)	7.27 <i>e</i>	1.09	0.8 <i>a</i>	3.1 <i>b</i>	15.3	34.9 <i>d</i>	84.4	1111.5 <i>bc</i>	7.6 <i>a</i>	5.7 <i>bc</i>	
3. KIO <sub>3</sub> (without N)	7.19 <i>de</i>	1.14	1.0 <i>a</i>	2.7 <i>b</i>	14.6	29.3 <i>b</i>	86.2	1104.5 <i>bc</i>	7.6 <i>a</i>	6.4 <i>c</i>	
4. Ca(NO <sub>3</sub> ) <sub>2</sub> +KI	7.14 <i>cd</i>	1.45	0.5 <i>a</i>	7.7 <i>c</i>	16.0	26.7 <i>ab</i>	82.8	1053.3 <i>abc</i>	5.9 <i>a</i>	4.7 <i>ab</i>	
5. Ca(NO <sub>3</sub> ) <sub>2</sub> +KIO <sub>3</sub>	7.06 <i>bc</i>	0.52	3.0 <i>b</i>	16.0 <i>d</i>	13.9	30.8 <i>bc</i>	79.9	1008.2 <i>ab</i>	7.7 <i>a</i>	6.2 <i>c</i>	
6. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +KI	7.01 <i>b</i>	0.90	0.7 <i>a</i>	8.4 <i>c</i>	16.4	35.8 <i>d</i>	89.6	1149.2 <i>c</i>	32.6 <i>b</i>	4.6 <i>a</i>	
7. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +KIO <sub>3</sub>	6.64 <i>a</i>	0.72	7.7 <i>c</i>	18.9 <i>e</i>	13.3	35.5 <i>d</i>	86.6	1083.2 <i>abc</i>	84.8 <i>c</i>	6.4 <i>c</i>	
Test <i>F</i>	*	n.s.	*	*	n.s.	*	n.s.	*	*	*	
(mg kg <sup>-1</sup> soil)											
	B	Cu	Fe	Mn	Zn	Mo	Al	Cd	Pb		
1. Control (without N and I)	1.3	6.63 <i>bc</i>	2073.6 <i>d</i>	239.8 <i>de</i>	38.9 <i>a</i>	1.33 <i>d</i>	1453.4 <i>d</i>	0.735 <i>ab</i>	39.1 <i>b</i>		
2. KI (without N)	1.3	5.99 <i>ab</i>	1920.1 <i>ab</i>	240.6 <i>de</i>	49.3 <i>b</i>	1.18 <i>ab</i>	1384.6 <i>a</i>	0.743 <i>abc</i>	74.7 <i>c</i>		
3. KIO <sub>3</sub> (without N)	1.9	6.82 <i>c</i>	1996.3 <i>c</i>	229.7 <i>b</i>	38.8 <i>a</i>	1.16 <i>a</i>	1406.1 <i>ab</i>	0.736 <i>ab</i>	21.7 <i>a</i>		
4. Ca(NO <sub>3</sub> ) <sub>2</sub> +KI	1.2	7.09 <i>c</i>	1882.8 <i>a</i>	236.3 <i>c</i>	39.2 <i>a</i>	1.19 <i>abc</i>	1403.6 <i>ab</i>	0.729 <i>b</i>	63.4 <i>c</i>		
5. Ca(NO <sub>3</sub> ) <sub>2</sub> +KIO <sub>3</sub>	1.2	5.96 <i>ab</i>	1935.6 <i>b</i>	221.7 <i>a</i>	39.0 <i>a</i>	1.22 <i>bc</i>	1422.5 <i>bc</i>	0.727 <i>a</i>	20.9 <i>a</i>		
6. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +KI	1.2	5.63 <i>a</i>	2035.5 <i>cd</i>	245.8 <i>e</i>	40.4 <i>a</i>	1.22 <i>c</i>	1436.3 <i>cd</i>	0.752 <i>bc</i>	21.8 <i>a</i>		
7. (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> +KIO <sub>3</sub>	1.0	6.40 <i>bc</i>	2150.1 <i>f</i>	262.1 <i>f</i>	38.9 <i>a</i>	1.38 <i>f</i>	1509.2 <i>f</i>	0.758 <i>c</i>	119.3 <i>d</i>		
Test <i>F</i>	n.s.	*	*	*	*	*	*	*	*		

Means followed by the same letters are not significantly different for  $P < 0.05$ .

\* means are significantly different, n.s. – not significant, y

both with and without N nutrition, resulted in a higher content of N-total in storage roots but had no influence on the level of this element in leaves. In the present work, soil application of KI without nitrogen, in comparison to the control and  $\text{KIO}_3$ , contributed to a higher content of N-total in carrot (Table 1). In the case of plants fertilized with nitrogen (both as calcium nitrate and ammonium sulphate), soil application of  $\text{KIO}_3$  (when compared to KI) significantly increased the level of N-total in carrot storage roots. The effects shown above could have been related to a higher concentration of mineral nitrogen in soil from these combinations (Table 2). The results indicate that the  $\text{IO}_3^-$  form of iodine most affected the ammonium nitrogen stabilization in soil, possibly by depressing nitrification processes – in combination with the ammonium sulphate application. Notably, the concentration of  $\text{N-NO}_3$  in soil fertilized with  $\text{Ca(NO}_3)_2 + \text{KIO}_3$  was only slightly lower when compared to the application of  $(\text{NH}_4)_2\text{SO}_4 + \text{KIO}_3$ . Moreover, the level of this N form in soil in the above combinations was distinctively higher when compared to KI fertilization along with the both tested nitrogen fertilizers. Iodine in the form of  $\text{IO}_3^-$  may have limited total denitrification of  $\text{NO}_3^-$  to  $\text{N}_2$ . It is worth mentioning that trace amounts of mineral nitrogen were found in soil after carrot cultivation in pots (SMOLEŇ et al. 2009a). In the available literature, no information can be on the influence of iodine (in  $\text{I}^-$  or  $\text{IO}_3^-$  form) on the conversion of mineral nitrogen in the soil environment. Therefore, it is difficult to compare our results with those reported by other authors.

In storage roots of plants grown without N, iodine application (in both forms: KI and  $\text{KIO}_3$ ) led to a significant increase in the P, K and Ca content as well as a reduction in the Fe concentration. It had no influence, however, on the accumulation of Mg, S, Cu, Mn, Zn, Mo, Al and Pb in carrot (Table 1). Changes in the K, Ca and Fe concentration in carrot observed in our trials were clearly attributable to the influence of KI and  $\text{KIO}_3$ , such as an elevated content of easily soluble forms of K and Ca, as well a reduced level of Fe in soil (Table 2). It should be added that in the study conducted by SMOLEŇ (2009), fertilization with  $\text{KIO}_3$  but without N led to higher accumulation of Al and Li as well as a reduced Cu content in carrot while the application of KI depressed the level of B, Fe, Ti and V in storage roots.

Interesting results were found with reference to iodine influence (in KI and  $\text{KIO}_3$  forms) on mineral composition of carrot roots in dependence on the type of nitrogen fertilizer (Table 1). A significant increase in K, Fe and Zn concentration in carrot was noted after using  $\text{Ca(NO}_3)_2 + \text{KIO}_3$  rather than  $\text{Ca(NO}_3)_2 + \text{KI}$ . In case of K and Fe, obtained results might have been related to higher level of easily soluble forms of these elements in soil (Table 2). In respect to plants fertilized with calcium nitrate, type of used iodine forms had no significant effect (when comparing KI and  $\text{KIO}_3$ ) on the content of: P, Mg, Ca, S, Na, B, Cu, Mn, Mo, Al, Cd and Pb in carrot storage roots.



Soil application of  $\text{KIO}_3$  (in comparison to KI) to plants fertilized with ammonium sulphate contributed to an increased concentration of P, K, Mg, S, Na, B, Cu, Fe, Mn, Zn, Al and Cd in carrot (Table 1). Among these elements, a significantly higher level of easily soluble forms of S, Na, Cu, Fe, Mn and Al was found in soil after cultivation of carrot fertilized with  $(\text{NH}_4)_2\text{SO}_4 + \text{KIO}_3$  (when compared to  $(\text{NH}_4)_2\text{SO}_4 + \text{KI}$ ), which could have directly affected its content in carrot roots. The concentrations of P, K, Mg, B, Zn and Cd in soil from these both combinations remained comparable.

The results of our experiment allow us to assume that an increased content of P, K, Mg, B, Zn and Cd in carrot fertilized with  $(\text{NH}_4)_2\text{SO}_4 + \text{KIO}_3$  could have been caused by the synergistic interaction of the  $\text{IO}_3^-$  form of iodine and  $\text{SO}_4^{2-}$  ions, affecting the uptake of these elements by plants. Furthermore, nitrogen fertilization in either form (calcium nitrate, ammonium sulphate) and application of iodate could have stimulated the uptake of zinc from soil. With these results, it is impossible to objectively claim whether higher accumulation of Zn in carrot was related to some synergism between  $\text{IO}_3^-$  and  $\text{NO}_3^-/\text{SO}_4^{2-}$  or with cations ( $\text{Ca}^{2+}$ ,  $\text{NH}_4^+$ ) introduced to soil with mineral fertilizers. Nonetheless, it is noteworthy that in the earlier pot experiment with carrot (SMOLEŇ 2009) no significant effect of KI and  $\text{KIO}_3$  application (with different N fertilization) on the Zn content in carrot was found. In the above study, nitrogen application, both in the  $\text{Ca}(\text{NO}_3)_2$  and  $(\text{NH}_4)_2\text{SO}_4$  forms, modified the influence of iodine on the uptake and accumulation of Ca, K, Mg, Na, P and S (SMOLEŇ et al. 2009a) as well as Al, B, Cd, Cr, Cu, Fe, Li, Ti and V (SMOLEŇ 2009) in comparison to the combinations without N fertilization.

In the present study, contrary results were obtained with respect to the effect of iodine application as KI and  $\text{KIO}_3$  (with different nitrogen fertilization) on mineral composition of storage roots in comparison to our previous experiments with pot cultivation of carrot (SMOLEŇ 2009, SMOLEŇ et al. 2009a). This inconsistency could be related to the different methods of carrot cultivation, particularly resulting from the limited capacity of growth by carrot roots in the pot experiment.

## CONCLUSIONS

1. In comparison to the control, a significant increase in the iodine content in carrot storage roots was found in all the combinations with iodine application in the form of KI.
2. The best effects of iodine biofortification of carrot was obtained through iodine application in the form of KI, particularly together with N fertilization with ammonium sulphate.

3. Iodine application as  $\text{KIO}_3$  (when compared to KI) significantly improved nitrogen utilization from mineral fertilizers by carrot plants.

4. The influence of the iodine form on the N-total content in carrot roots was varied, depending on whether cultivation was carried out without N fertilization or using ammonium sulphate and calcium nitrate.

5. In the combinations without N nutrition, iodine application (both as KI and  $\text{KIO}_3$ ) contributed to a significant increase in the P, K and Ca content and a reduction in the Fe concentration but had no effect on the Mg, S, Cu, Mn, Zn, Mo, Al and Pb accumulation in carrot storage roots.

6.  $\text{KIO}_3$  application, when compared to KI, to plants fertilized with ammonium sulphate increased the concentration of: N, P, K, Mg, S, Na, B, Cu, Fe, Mn, Zn, Al and Cd in carrot. In the case of fertilization with calcium nitrate,  $\text{KIO}_3$  treatment led to higher levels of N, K, Fe and Zn in carrot storage roots than application of KI.

7. In the combinations without N fertilization, changes in the carrot content of K, Ca and Fe were related to the influence of iodine on the availability of easily soluble forms of these elements in soil. In the case of  $\text{Ca}(\text{NO}_3)_2$  application, it caused accumulation of K and Fe. When  $(\text{NH}_4)_2\text{SO}_4$  was used, the concentrations of S, Na, Cu, Fe, Mn and Al in carrot roots were all affected by some interaction of iodine with these elements in soil.

8. Iodine application in the form of KI and  $\text{KIO}_3$  together with N fertilization had a variable influence on soil pH as well as the content of N- $\text{NH}_4$ , N- $\text{NO}_3$ , K, S, Na, Cu, Fe, Mn, Mo, Al, Cd and Pb in soil after carrot cultivation.

## REFERENCES

- ALTMOK S., SOZUDOGRU-OK S., HALILOVA H. 2003. *Effect of iodine treatments on forage yields of alfalfa*. Com. Soil Sci. Plant Anal., 34(1-2): 55-64.
- BORST PAUWELS G.W. F.H. 1961. *Iodine as a micronutrient for plants*. Plant Soil, 14: 665-671.
- GORLACH E., CURYLO T., GAMBUŚ F., GRZYWNOWICZ I., JASIEWICZ C., KOPEĆ M., MAZUR B., OLKUŚNIK S., ROGÓZ A., WIŚNIEWSKA-KIELIAN B. 1999. *Przewodnik do ćwiczeń z chemii rolnej [A guidebook to experimentation in agricultural chemistry]*. Wyd. AR w Krakowie. (in Polish)
- KABATA-PENDIAS A., MUKHERJEE A.B. 2007. *Trace elements from soil to human*. Springer.
- NOWOSIELSKI O. 1988. *The rules in development of fertilizing strategies in horticulture*. PWRiL, Warsaw. (in Polish)
- PASŁAWSKI P., MIGASZEWSKI Z.M. 2006. *The quality of element determinations in plant materials by instrumental methods*. Part I. Pol. J. Environ. Stud., 15(2a) 154-164.
- PERSSON, J.A., WENNERHOLM M. 1999. *A guide of the mineralization with Kjeldahl method*. Warsaw, Poland, Labconsult. (in Polish).
- PN-EN ISO 11732:2005 (U). *Water quality. Determination of ammonium nitrogen. Method involving flow analysis (CFA and FIA) and spectrometric detection*. (in Polish).
- PN-EN ISO 13395:2001. *Water quality – Determination of nitrite nitrogen and nitrate and the sum of both by flow analysis (CFA and FIA) and spectrometric detection*. (in Polish).

- prEN 15111: R2-P5-F01 – 2006 (E). *Artykuły żywnościowe – Oznaczenie pierwiastków śladowych – Oznaczenie zawartości jodu metodą ICP MS (spektrometria masowa z plazmą wzbudzoną indukcyjnie) [Food products – Determination of trace elements – Determination of iodine with the ICP MS method (mass spectrophotometry with inductively coupled plasma)]*. (in Polish)
- SMITH G.S., MIDDLETON K.R. 1982. *Effect of sodium iodide on growth and chemical composition of lucerne and ryegrass* Fert. Res., 3: 25-36.
- SMOLEŃ S. 2009. *Wpływ nawożenia jodem i azotem na skład mineralny marchwi [Effect of iodine and nitrogen fertilization on chemical composition of carrot]*. Ochr. Środ. Zasob. Nat., 40: 270-277. (in Polish)
- SMOLEŃ S., LEDWOŻYŃ I., STRZETELSKI P., SADY W., ROŻEK S. 2009a. *Wpływ nawożenia jodem i azotem na efektywność biofortyfikacji w jod oraz na jakość biologiczną marchwi [Effect of iodine and nitrogen fertilization on effectiveness of biofortification with iodine and on the biological quality of carrot]*. Ochr. Środ. Zasob. Nat., 40: 313-320. (in Polish)
- SMOLEŃ S., SADY W., STRZETELSKI P., ROŻEK S., LEDWOŻYŃ I. 2009. *Wpływ nawożenia jodem i azotem na wielkość i jakość plonu w marchwi [Effect of iodine and nitrogen fertilization on volume and quality of yield produced by carrot]*. Ochr. Środ. Zasob. Nat., 40: 286-292. (in Polish)
- STRZETELSKI P. 2005. *Występowanie i przemieszczanie jodu w systemie gleba-roślina [Presence and migration of iodine in the soil-plant system]*. Post. Nauk Rol., 6: 85-100. (in Polish)
- STRZETELSKI P., SMOLEŃ S., ROŻEK S., SADY W. 2010. *The effect of diverse iodine fertilization on nitrate accumulation and content of selected compounds in radish plants (Raphanus sativus L.)*. Acta Scient. Pol. Hort. Cult., 9 (2): 65-73.
- WHITE P.J., BROADLEY M.R. 2005. *Biofortifying crops with essential mineral elements*. Trends Plant Sci., 10(12): 586-593.
- WHITE P.J., BROADLEY M.R. 2009. *Biofortification of crops with seven mineral elements often lacking in human diets – iron, zinc, copper, calcium, magnesium, selenium and iodine*. New Phytol., 182(1): 49-84.
- WINGER R.J., KÖNIG J., HOUSE D.A. 2008. *Technological issues associated with iodine fortification of foods*. Trends Food Sci. Tech., 19: 94-101.
- YANG X-E., CHEN W-R., FENG Y. 2007. *Improving human micronutrient nutrition through biofortification in the soil-plant system: China as a case study*. Environ. Geochem. Health., 29(5): 413-28.
- ZHAO F.-J., McGRATH S.P. 2009. *Biofortification and phytoremediation*. Curr. Opin. Plant Biol., 12: 373-380.

