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IODINE AND SELENIUM BIOFORTIFICATION OF LETTUCE (*Lactuca sativa* L.) BY SOIL FERTILIZATION WITH VARIOUS COMPOUNDS OF THESE ELEMENTS

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Abstract. Relatively little is known on the interaction between iodine and selenium in plants. It may become a drawback in developing agrotechnical rules of plant biofortification with these elements. The aim of the study was to determine the influence of soil fertilization with various forms of iodine (I^- and IO_3^-) and selenium (SeO_3^{2-} and SeO_4^{2-}) on yield, biofortification efficiency and selected chemical properties of lettuce plants. The study (conducted in 2012–2014) included soil fertilization of lettuce cv. 'Valeska' in the following combinations: control (without iodine and selenium fertilization), KI, KIO_3, Na_2SeO_4, Na_2SeO_3, KI + Na_2SeO_4, KIO_3 + Na_2SeO_4, KI + Na_2SeO_3, KIO_3 + Na_2SeO_3. Iodine and selenium were applied twice: before sowing and as a top-dressing in a total dose of 5 kg $I\cdot ha^{-1}$ and 1 kg $Se\cdot ha^{-1}$. Only the application of Na_2SeO_4 (individually or together with iodine) exhibited strong toxic effect on plants which was accompanied by the highest accumulation of Se, selenomethionine (SeMet) and selenocysteine (SeCys) in lettuce. The accumulation of I and Se in lettuce was respectively higher after fertilization KI than KIO_3 and Na_2SeO_4 than Na_2SeO_3. Simultaneous application of iodine and selenium decreased the level of Se, SeMet and SeCys in lettuce – particularly in the combination with KIO_3 + Na_2SeO_3. Simultaneous application of KI with both forms of selenium decreased iodine content in lettuce as related to the treatment with KI alone. In the case of lettuce from the combinations with KIO_3, KIO_3 + Na_2SeO_4 and KIO_3 + Na_2SeO_3, comparable results of iodine concentration were obtained.

Key words: biofortification, biological quality, selenomethionine, selenocysteine, cold vapor generation of iodine

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INTRODUCTION

Almost two-third of human population suffers from diseases related to insufficient supply of iodine and selenium in the diet. One of the easiest and most effective methods to counteract this problem is biofortification of crop plants with mineral nutrients [White and Broadley 2009, Przybysz et al. 2015]. Simultaneous biofortification with iodine and selenium may be however problematic as both elements are not essential mineral nutrients for plants [Kopsell and Kopsell 2007, Kabata-Pendias 2011]. Yet, selenium is included to the group of "beneficial elements" [Kopsell and Kopsell 2007].

No information is available on nationwide agrotechnical programmes of iodine biofortification of crop plants as similar to those conducted with selenium in Finland or Malawi [Eurola et al. 2003, Chilimba et al. 2012]. One of the few examples includes iodination of irrigation water using KIO_3 in southern Xinjiang Province of China. With low economic costs, it increased the content of available iodine in soils and consequently in the food [Ren et al. 2008].

In the recent years WHO presented the "Global Strategy on Diet, Physical Activity and Health" aimed, amongst others, at limiting the consumption of table salt with searching for alternative sources of iodine in the human diet [WHO 2004, 2014]. It seems, however, that WHO does not perceive the huge potential of iodine-enriched plants [WHO 2014], even though numerous studies on iodine biofortification of crop plants have already been conducted [*i.e.* Blasco et al. 2010, Voogt et al. 2010].

In lettuce cultivation in hydroponics, soilless systems or pot experiments the influence of SeO_3^{2-} and SeO_4^{2-} on: biomass, selenium accumulation, antioxidant activity, the content of secondary plant metabolites [Ríos et al. 2008, Ríos et al. 2010] or nitrogen metabolism of plants was described [Ríos et al. 2010]. Also the effect of iodine (I^- and IO_3^-) on basic physiological and biochemical processes in lettuce grown in hydroponics (in greenhouses or growth chambers) was tested [Blasco et al. 2010, Voogt and Jackson 2010, Voogt et al. 2010].

The problem of interaction between iodine and selenium in plants is not yet sufficiently diagnosed. Few studies on simultaneous application of iodine and selenium in spinach [Zhu et al. 2004] or lettuce cultivation [Smoleń et al. 2014] were conducted in hydroponics. With the exception of one-year studies conducted by Mao et al. [2014] with Se + Zn + I fertilization during the cultivation of wheat, maize, soybean, potato, canola and cabbage no other works focused on simultaneous application of various iodine and selenium compounds in field studies are available.

Lettuce belongs to model species used in some primary research [Dzida et al. 2012 a, b, Pitura and Michałojć 2012, Borowski et al. 2014], including iodine [Blasco et al. 2010] and selenium biofortification [Ramos et al. 2010, Ríos et al. 2008, 2010].

The aim of the study was to determine the influence of soil fertilization with various forms of iodine (I^- and IO_3^-) and selenium (SeO_3^{2-} and SeO_4^{2-}) on yield, biofortification efficiency and selected chemical properties of lettuce plants (*Lactuca sativa* L.).

MATERIAL AND METHODS

Plant material and treatments. In the years 2012–2014, a field study with lettuce cv. 'Valeska' cultivation was conducted in the Experimental Station (50°07'910 N, 19°84'764 E) of University of Agriculture in Krakow, Poland. Each year, lettuce was grown in spring on various plots of the same field.

Lettuce was cultivated on heavy soil (heavy clay: 24% sand, 23% silt and 53% loam). Chemical properties of soil in the subsequent years are presented in table 1. Lettuce was sown in the greenhouse in the first decade of March. Seedlings were planted into soil on 18, 23 and 15 April in the subsequent years in rows 30 cm apart with 30 cm spacing.

The study included soil fertilization with iodine and selenium in the following combinations: 1. Control, 2. KI, 3. KIO_3, 4. Na_2SeO_4, 5. Na_2SeO_3, 6. KI + Na_2SeO_4, 7. KIO_3 + Na_2SeO_4, 8. KI + Na_2SeO_3, 9. KIO_3 + Na_2SeO_3. Iodine and selenium were applied twice: before planting and as a top-dressing in the early stage of head formation (first decade of May; date indicated at fig. 1), each in a dose of 2.5 kg $I \cdot ha^{-1}$ + 0.5 kg $Se \cdot ha^{-1}$. Total amount of iodine and selenium introduced into the soil was 5 kg $I \cdot ha^{-1}$ and 1 kg $Se \cdot ha^{-1}$, respectively. Iodine and selenium were applied as KI and KIO_3 (Avantor Performance Materials, Poland) as well as Na_2SeO_4 and Na_2SeO_3 (Sigma-Aldrich, Germany) pure for analysis.

One day prior to lettuce planting (date indicated at fig. 1) pre-sowing fertilization with N, P and K was conducted (along with iodine and selenium application) based on the results of soil chemical analysis in order to supplement nutrient deficiencies to the level optimal for lettuce: N – 100, P – 70 and K – 200 $mg \cdot dm^{-3}$ of soil. Nitrogen was applied as ammonium nitrate in a dose of 50 mg $N \cdot dm^{-3}$ soil which is 50% N. The second dose of nitrogen was introduced with top-dressing application of iodine and selenium and followed by plant watering with approximately 15 mm of precipitation. Fertilization with other macronutrients was not applied as its content in soil covered lettuce requirements (tab. 1).

The experiment was arranged in a split-plot design. Each experimental treatment was randomized in four replications on 5 m × 1.5 m (7.5 m^2) plots – 64 plants per one plot. The total area under the experiment was 270 m^2.

During harvest (30 May, 11 and 3 June in the subsequent years), yield was assessed by weighing sixteen lettuce heads from each plot. Eight lettuce heads were collected for further analysis as well as soil samples from 0–30 cm layer, individually for each treatment.

Plant analysis. Dry matter content in fresh lettuce samples was assayed at 105°C. After preparing ethanolic extracts of plant material, total content of phenolic compounds was determined using Folin-Ciocalteau reagent [Swain and Hillis 1959]. Additionally, lettuce leaves were dried at 70°C in a laboratory dryer with forced air circulation and ground in variable speed rotor mill Pulverisette 14, FRITSCH using 0.5 mm sieve. Thus-prepared samples were subsequently analyzed with respect to the content of iodine and selenium by ICP-OES technique (two separate procedures) as well as selenomethionine, selenocysteine, inorganic (I^-, IO_3^-, SeO_4^{2-}, SeO_3^{2-}) and organic ions (oxalates and citrates) using capillary electrophoresis (CE).

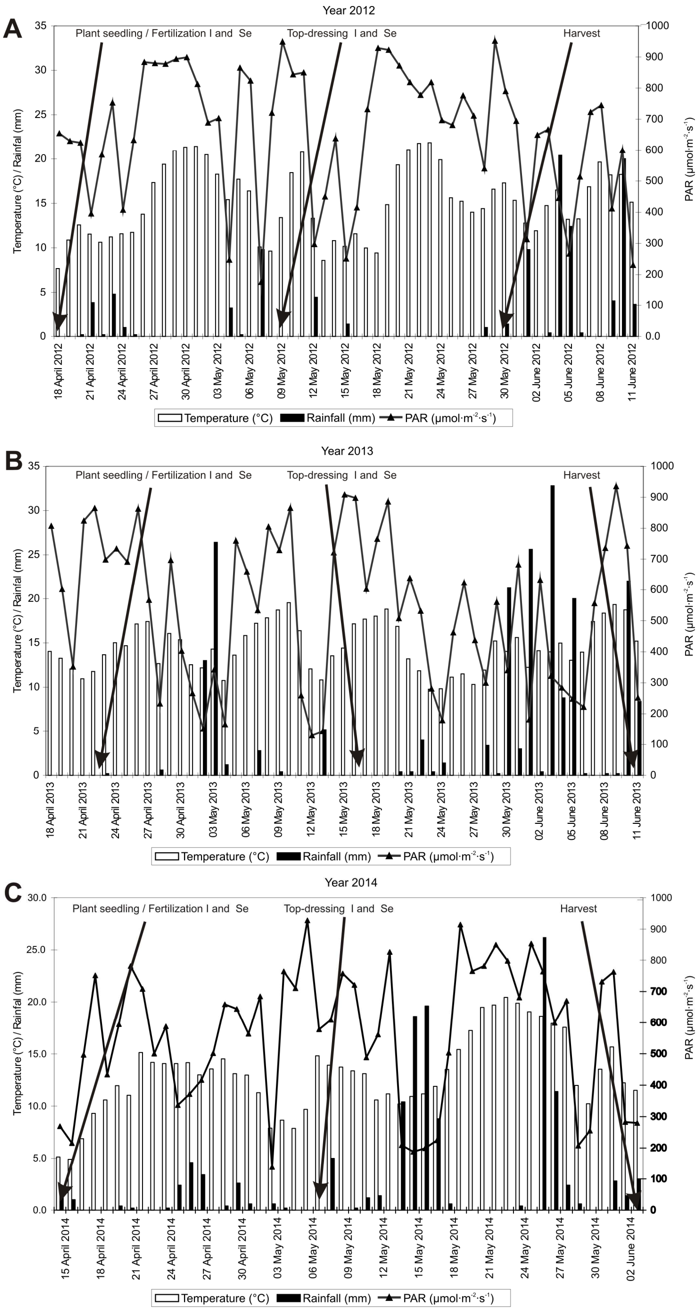

Fig. 1. Meteorological data for lettuce cultivation in 2012 (A), 2013 (B) and 2014 (C). Dates of: lettuce planting, pre-sowing and top dressing application of I and Se as well as harvest are indicated

Table 1. Selected chemical properties of 0–30 cm soil layer prior to the experiment in 2012–2014

Parameter	Year		
	2012	2013	2014
pH_{H_2O}	6.72	7.45	7.19
EC (mS cm^{-1})	0.10	0.11	0.10
Eh (mV)	276.0	268.0	189.3
$N\text{-}NH_4 + N\text{-}NO_3$ (mg·dm^{-3})^1	13.4	12.7	8.4
P (mg·dm^{-3})	32.7	12.8	59.5
K (mg·dm^{-3})	181.4	139.1	202.3
Ca (mg·dm^{-3})	2 089.3	1 651.8	1 011.0
Mg (mg·dm^{-3})	194.2	182.8	114.0
Na (mg·dm^{-3})	8.6	23.5	2.9
S (mg·kg^{-1})	41.4	42.9	22.1
I (mg·kg^{-1})	1.87	1.93	1.80
Se (mg·kg^{-1})	0.90	0.88	0.92
Organic matter (%)	2.33	2.56	2.33
Cation exchange capacity (CEC cmol kg^{-1})	8.32	9.58	8.48
Saturation of the sorption complex with alkaline elements (%)	92.7	88.2	85.8

1 – units: mg·dm^{-3} and mg·kg^{-1} depend on applied analytical method

Determination of iodine and selenium content after TMAH (tetramethylammonium hydroxide) extraction. 0.5 g of air-dried leaf samples, 10 cm^3 of double-distilled water and 1 cm^3 of 25% TMAH were put into 30 cm^3 falcon tubes. After mixing, samples were incubated for 3 hours at 90°C. After incubation, samples were cooled to the temperature of approximately 20°C and filled to 30 cm^3 with double-distilled water. After mixing, samples were centrifuged for 15 minutes at 4 500 rpm. The measurements using ICP-OES spectrometer were conducted in the supernatant (without its decanting) [PN-EN 15111, 2008].

Additionally, analyses of iodine using cold vapor I_2 generation technique (CVG) [Vtorushina et al. 2008, 2009] and selenium after sample digestion in 65% HNO_3 [Pasławski and Migaszewski 2006] were performed. For CVG: digestion of 0.5 g air-dried plant samples in the mixture of 10 cm^3 65% HNO_3 and 0.8 cm^3 70% $HClO_4$ was conducted in the CEM MARS-5 Xpress microwave system using teflon vessels. The process consisted of four steps with gradual increase of temperature: 60, 80, 100 (each step included 10 min of warming plus 5 min of maintaining set temperature) and 130°C (10 min + 15 min). After the digestion, solutions were transferred into the volume of 25 cm^3 with redistilled water. Measurements using CVG technique required the application of the prototype gas/liquid separator for ICP-OES Prodigy spectrometer (Supplementary material Photo S1), which was constructed according to Vtorushina et al. [2008].

For selenium analysis, 0.5 g of air-dried plant samples was digested at 200°C (15 min of warming plus 15 min of maintaining set temperature) in 10 cm^3 65% super pure HNO_3 using microwave system CEM MARS-5 Xpress. Samples were then transferred to the final volume of 25 cm^3 using double-distilled water and analyzed with ICP-OES spectrometer.

Determination of the content of selenomethionine (SeMet) and selenocysteine (SeCys). In 30 cm^3 falcon tubes, 5 ml of solution containing 40 mg protease and 20 mg lipase in demineralized water were added to 0.25 g of air-dried leaves. Samples were incubated for 16 h at 20°C and centrifuged for 15 min at 4500 rpm [Zhao et al. 2011]. The aliquots of 0.5 cm^3 of supernatants were transferred into 1.5 cm^3 Eppendorf tubes and centrifuged for 10 min at 10 000 rpm. Measurements of SeMet and SeCys content were conducted directly in supernatants using capillary electrophoresis system Capel 105 M (Lumex, Russia) with UV detection at 254 nm. Capillaries of i.d. 75 μm, o.d. 365 μm and total length of 50 cm were used. A negative power supply of -16 kV was applied. The running buffer solution was prepared as proposed by Zhao et al. [2011] containing 30 mM NaH_2PO_4, 15 mM $Na_2B_4O_7$ and 0.2 mM CTAB (cetyltrimethylammonium bromide) (pH 8.80). Standards of L(+)-selenomethionine and L-selenocysteine (Acros Organics) were used for CE calibration.

Analysis of the content of I^-, IO_3^-, SeO_4^{2-}, SeO_3^{2-}, oxalates and citrates. 0.1 g of air-dried plant samples with 20 cm^3 of extraction solution containing: 4 cm^3 25% TMAH and 10 cm^3 0.1 M NaOH in 1 dm^3 of demineralized water were put into 30 cm^3 falcon tubes. After mixing, samples were incubated for 1 hour at 90°C, cooled to the temperature of approximately 20°C, mixed thoroughly and centrifuged for 15 min at 4 500 rpm. The supernatants were filtered through a 0.25 μm cellulose acetate membrane filters and analyzed using PA 800 Plus capillary electrophoresis system (Beckman Coulter, USA*)* with DAD detection. Capillaries of i.d. 75 μm, o.d. 365μm and total length of 60 cm (50 cm to detector) were used. A negative power supply of -25 kV was applied. Commercial kit Anions-2 Kit (Analis Belgium) was used for ion analysis with the following standards for CE calibration: KI, KIO_3, Na_2SeO_3, Na_2SeO_4 (Sigma Aldrich) as well as oxalate acid and citrate acid (POCH Poland).

The conduction of oxalates and citrates analyses resulted from the possibility of its determination along with I^-, IO_3^-, SeO_4^{2-}, SeO_3^{2-} during the same separation. It reduced the costs and times of analyzing these compounds by other methods. Oxalates and citrates are the most abundant organic acid derivatives found in plant cells. Their content in plants is dependent, among others, on calcium nutrition [Wińska-Krysiak 2006]. The level of citrate synthase expression (formally the first enzyme in that cycle, responsible for the formation of citrate) is directly dependent on the growth of plants [Koyama et al. 1999]. The accumulation of citrates in plants is correlated with the H+ efflux, possibly from the action of H+ -ATPase on the plasma membrane [Ohno et al. 2003].

Soil analysis. Soil samples were dried at 70°C in a laboratory dryer with forced air circulation, ground in a mortar and sieved through 1 mm sieve. 2.5 g of soil samples were put into 30 cm^3 falcon tubes, then 10 cm^3 of double-distilled water and 1 cm^3 of 25% TAMH were added. Further steps were as described for I and Se determination in TMAH in lettuce leaves. The method described above is our own modified procedure [Smoleń et al. 2016] for the determination of total I [Yamada et al. 1996] and I and Se [McNally 2011] content in soil using TMAH. The modification included the application of higher temperature (90, not 70°C) and omitting sample filtration after centrifugation. Filtration of samples leads to losses of analyzed elements. The temperature of 90°C is recommended for I determination after sample incubation with TMAH according to PN-EN 15111 (2008).

ICP-OES spectrometer settings for iodine and selenium determination. The analysis of iodine and selenium content in lettuce and soil samples was conducted using ICP-OES Prodigy spectrometer (Teledyne Leeman Labs USA). Calibration of the instrument was performed maintaining the same matrix as for analyzed samples.

For iodine determination in lettuce samples after alkaline extraction (using TMAH) more sensitive spectral line I-206.163 was applied, while for soil – I-183.038 nm, the latter one being not affected by Cr and Zn spectral interferences. Selenium content in both lettuce and soil samples was analyzed using Se-169.090 nm line.

For iodine determination using CVG technique, the most sensitive line of iodine I-178.276 nm was chosen as for this method no interferences (including these from phosphorus) affect the reading [Vtorushina et al. 2009].

Meteorological data. Each year lettuce was cultivated from the end of April until the end of May or first days of June (43, 55 and 50 days in 2012, 2013 and 2014, respectively – fig. 1 A–C). Average daily air temperature in May was 15.6, 14.0 and 13.8°C in subsequent years. In comparison, in the years 1971–2000 average monthly air temperature was: 8.0°C in April, 13.4°C in May and 16.2°C in June [GUS 2005].

Average daily value of PAR radiation in the period from lettuce planting in field to the harvest was: 656.7, 537.8 and 562.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The total amount of rainfall was 31.6, 203.8 and 132.2 mm in 2012, 2013 and 2014, respectively (fig. 1 – data from HOBO Weather Station). In 2013 and 2014 heavy rainfall occurred few days before lettuce harvest. In the years 1971–2000 average monthly rainfall in the area under study was: 50 mm in April, 74 mm in May and 94 mm in June [GUS 2005].

The period of lettuce cultivation in 2012 was characterized by the most unfavorable weather conditions as compared to respective time in 2013 and 2014. In that year the lowest amount of rainfall as well as the highest values of average daily air temperature and PAR radiation were noted.

Data analysis. Obtained data were subjected to analysis of two-way variance (treatment × year of study) using ANOVA module of Statistica 10.0 PL. To determine the significance between means the Tukey's test was used. The significance was declared at $P < 0.05$.

RESULTS

Yield and dry matter content. A significant influence of iodine and selenium application on total yield and dry matter content in lettuce was noted (tab. 2) – however, only the level of dry matter was affected by the treatment × year of study interaction (figs 2 A and B).

In comparison to the control and other combinations, application of Na_2SeO_4 (both alone and with KI or KIO_3) led to a decrease in yield (in each year of the study; tab. 2 and fig. 2A) as well as an increase in dry matter content in lettuce (means for 2012–2014; tab. 2). In the latter case, various yet statistically significant quantitative relations between tested combinations were found in individual years of the study. However, each year the content of dry matter was higher in lettuce grown on soil fertilized with Na_2SeO_4, KI+Na_2SeO_4 and KIO_3+Na_2SeO_4 as compared to other treatments.

Table 2. Yield and the content of dry matter, iodine and selenium (determined in TMAH, CVG and HNO_3) as well as SeMet and SeCys in lettuce – means from 2012–2014

Treatment (n = 12, ±s.e.)	Yield (t·ha^{-1})	D.M. (% d.w.)	Iodine		Selenium		Seleno amino acid		Share of Se from aminoacids (SeMet and SeCys) in the total Se content (means from Se analyzed in TMAH and HNO_3)
			(mg I·kg^{-1} d.w.)		(mg Se·kg^{-1} d.w.)				
			in TMAH	by CVG[1]	in TMAH	in HNO_3	(mg SeMet·/ kg^{-1} d.w.)	(mg SeCys·/ kg^{-1} d.w.)	
Control	48.3 ± 3.0^{b}	4.45 ± 0.37^{a}	1.5 ± 0.1^{a}	1.4 ± 0.1^{a}	2.6 ± 0.5^{a}	3.5 ± 0.9^{a}	3.1 ± 0.8^{a}	1.7 ± 0.2^{a}	67.1%
KI	49.6 ± 1.9^{b}	4.42 ± 0.32^{a}	6.8 ± 0.6^{d}	5.6 ± 0.5^{e}	1.7 ± 0.3^{a}	1.9 ± 0.1^{a}	2.3 ± 0.2^{a}	1.6 ± 0.3^{a}	89.3%
KIO_3	48.5 ± 2.1^{b}	4.45 ± 0.34^{a}	3.8 ± 0.3^{b}	4.0 ± 0.3^{b}	2.7 ± 0.6^{a}	2.7 ± 0.3^{a}	3.1 ± 0.3^{a}	3.0 ± 0.4^{a}	98.4%
Na_2SeO_4	30.1 ± 2.8^{a}	5.18 ± 0.31^{c}	1.3 ± 0.3^{a}	1.5 ± 0.1^{a}	142.4 ± 25.0^{e}	110.6 ± 25.1^{e}	155.6 ± 35.5^{f}	100.5 ± 28.9^{f}	86.9%
Na_2SeO_3	44.2 ± 2.0^{b}	4.48 ± 0.41^{a}	0.9 ± 0.1^{a}	1.5 ± 0.1^{a}	17.0 ± 4.8^{c}	16.4 ± 4.4^{c}	23.3 ± 5.9^{c}	14.7 ± 4.6^{c}	97.5%
$KI+Na_2SeO_4$	29.0 ± 2.7^{a}	5.09 ± 0.32^{c}	5.2 ± 0.8^{c}	4.6 ± 0.3^{cd}	116.0 ± 19.4^{d}	82.7 ± 14.5^{d}	107.9 ± 10.6^{d}	74.0 ± 16.0^{e}	78.7%
$KIO_3+Na_2SeO_4$	30.0 ± 2.9^{a}	5.23 ± 0.38^{c}	3.6 ± 0.3^{b}	4.2 ± 0.3^{bc}	118.8 ± 19.2^{d}	86.7 ± 15.4^{d}	147.4 ± 23.1^{e}	49.2 ± 8.6^{d}	80.3%
$KI+Na_2SeO_3$	44.7 ± 2.2^{b}	4.63 ± 0.40^{b}	5.2 ± 0.5^{c}	4.7 ± 0.2^{d}	15.2 ± 3.7^{c}	13.5 ± 3.9^{c}	12.9 ± 2.1^{b}	5.1 ± 0.4^{b}	52.9%
$KIO_3+Na_2SeO_3$	46.8 ± 1.7^{b}	4.48 ± 0.34^{a}	3.9 ± 0.4^{b}	4.6 ± 0.1^{cd}	10.7 ± 2.7^{b}	9.4 ± 2.6^{b}	8.0 ± 0.7^{b}	9.8 ± 2.1^{b}	77.9%

1 – CVG (cold vapor generation technique). D.M. Dry matter. Means followed by the same letters are not significantly different for $p < 0.05$;
s.e. – standard error

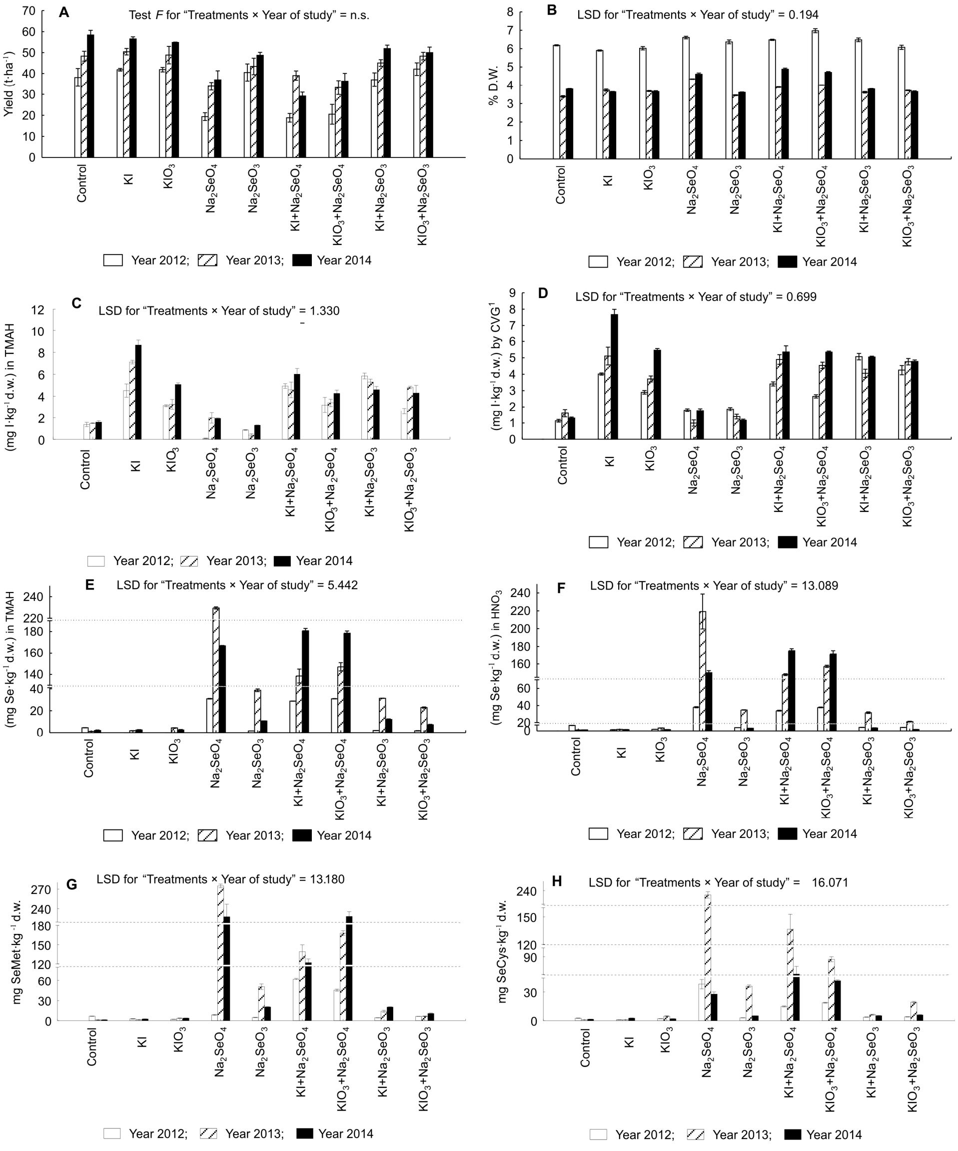

Fig. 2. Yield (A) and the content of dry matter (B), iodine (C and D) and selenium (E and F) – determined in TMAH (C and E), CVG (D) and HNO_3 (F) – as well as SeMet (G) and SeCys (H) in lettuce in each year of the study (2012–2014) depending on applied I and Se fertilization. Bars indicate standard error (n = 4)

Efficiency of iodine and selenium biofortification. The level of iodine and selenium bioaccumulation in lettuce was significantly affected by applied fertilization with these elements as well as soil and weather conditions in each year of cultivation (significant treatment × year of study interaction). Determined values of iodine and selenium concentration in lettuce depended also on applied method of sample preparation prior to the analysis (tab. 2 and fig. 2 C–F). Generally, soil fertilization with iodine and selenium increased the concentration of these elements in lettuce heads.

Analyzing the mean values from 2012–2014, it turned out that the efficiency of iodine biofortification of lettuce (determined in TMAH and by CVG) was higher after soil fertilization with KI rather than KIO_3 (tab. 2, figs 2 C and D). Simultaneous application of KI with both forms of selenium decreased iodine content in lettuce as related to the treatment with KI alone. Presented results concerning iodine biofortification of lettuce were however significantly affected by soil and weather conditions in individual years (figs 2 C and D).

In lettuce grown on soil fertilized with KI, KI + Na_2SeO_4 and KI + Na_2SeO_3, higher values of iodine content were obtained after sample extraction with TMAH than the application of CVG technique (tab. 2). This relation was mainly observed in 2012 while in other years application of CVG allowed to provide higher or comparable values of iodine to those noted for TMAH (figs 2 C and D). In the case of lettuce from the combinations with KIO_3, KIO_3 + Na_2SeO_4 and KIO_3 + Na_2SeO_3 comparable results of iodine concentration were obtained in each year irrespective of applied analytical procedure (tab. 2, figs 2 C and D).

With respect to selenium content, lettuce grown on soil fertilized with Na_2SeO_4 (alone or together with KI or KIO_3) contained several dozen-fold more Se than plants from the combinations with Na_2SeO_3 applied alone or with iodine in both forms (tab. 2, figs 2 E and F). Lettuce plants from the combinations with KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 accumulated significantly less Se than those from the combination with Na_2SeO_4 applied alone. The described relations were mainly affected by the results obtained in 2013 (fig. 2 E and F). In that year lettuce grown on soil fertilized with Na_2SeO_4 contained the highest level of selenium from all tested combinations and additional application of KI or KIO_3 significantly decreased its accumulation. On the other hand, simultaneous application of iodine and Na_2SeO_4 increased selenium concentration in lettuce in 2014 – especially when analyzed in TMAH.

For Na_2SeO_3 treatment, the additional application of KIO_3 (but no KI) decreased selenium content in lettuce (for selenium determination in TMAH and HNO_3 – tab. 2). Such relation was noted in 2013 and 2014 but not in 2012 (figs 2 E and F).

It is worth to underline that soil fertilization with Na_2SeO_4 and Na_2SeO_3 did not significantly affect iodine accumulation in lettuce when compared to the control (tab. 2). Also the application of KI and KIO_3 did not change selenium content in lettuce.

A positive value of correlation coefficient for various methods of sample preparation were noted for iodine r = 0.85 for $I_{in\ TMAH} \times I_{by\ CVG}$ and selenium content in lettuce r = 0.86 for $Se_{in\ TMAH} \times Se_{in\ HNO_3}$.

It was not possible to determine the content of: I^-, IO_3^-, SeO_4^{2-}, SeO_3^{2-} in lettuce heads by capillary electrophoresis using Anions-2 Kit and DAD detection – the level of these ions were below limits of its detection by PA 800 Plus instrument. Another model of CE – Capel 105 M with UV detection was additionally tested using running buffer solutions and capillaries as chosen for the analysis of SeMet and SeCys but with lower voltage: -4 kV at 195 nm. That analytical approach allowed to separate the peaks of individual ions from interferences (in samples with added standard) yet the sensitivity of the instrument was still too low to reliably determine its level in plant material.

The content of SeMet and SeCys. Soil fertilization with Na_2SeO_4 (also together with KI or KIO_3) significantly increased the accumulation of SeMet and SeCys in lettuce plants as compared to Na_2SeO_3 applied alone or with iodine (tab. 2). Simultaneous application of iodine and selenium in all tested forms decreased the content of these two seleno amino acids in lettuce when compared to combinations treated with selenium alone (tab. 2). These relations were noted in each year of the study (figs 2 G and H). Generally, the lowest level of SeMet and SeCys in lettuce heads was noted in 2012.

Soil fertilization with $KI+Na_2SeO_4$ more significantly decreased the accumulation of SeMet, while $KIO_3 + Na_2SeO_4$ treatment reduced SeCys level in lettuce as compared to the application of Na_2SeO_4 alone (tab. 2). On the other hand, additional application of iodine as KI or KIO_3 comparably reduced the level of SeMet, and to a lesser extent – of SeCys in lettuce as compared to plants from the combination with Na_2SeO_3 alone. Presented relations concerning negative impact of simultaneous application of iodine and selenium in tested forms on SeMet accumulation were observed in 2013 and 2014 but not in 2012. In the case of SeCys, this observation was valid only in 2013.

In comparison to the control, soil fertilization only with KI or KIO_3 had no significant effect on the content of SeMet and SeCys in lettuce (tab. 2, figs 2 G and H). The average share of both seleno amino acids in the total selenium content in lettuce ranged between 52.9% in the combination with $KI + Na_2SeO_3$ and 98.4% for KIO_3 fertilization (tab. 2).

The content of phenolic compounds, oxalates and citrates. A statistically significant influence of tested combinations as well as treatment × year of study interaction was noted with respect to the level of phenolic compounds, oxalates and citrates in lettuce (tab. 3 and figs 3 A-C). The highest content of phenolics was noted in lettuce grown on soil fertilized with Na_2SeO_4 alone or together with iodine (tab. 3) – most distinctively in 2012 (fig. 3 A). The greatest accumulation of oxalates was found in lettuce heads grown on soil treated with KI (tab. 3) – largely affected by the results obtained in 2013 (fig. 3 B). In each year of the study, soil fertilization with Na_2SeO_3, $KI + Na_2SeO_3$ and $KIO_3 + Na_2SeO_3$ significantly reduced the content of citrates in lettuce as compared to the control combination (tab. 3, fig. 3 C). Additionally, after soil fertilization with $KIO_3 + Na_2SeO_3$ lower accumulation of phenolic compounds was observed when compared to the control (tab. 3, fig. 3 A).

The content of iodine and selenium in soil after lettuce harvest. The content of iodine and selenium in soil after lettuce cultivation was substantially modified by applied fertilization with various compounds containing these elements (means from the three years of the study − tab. 4) as well as treatment × year of study interaction (figs 4 A and B). In combinations with iodine (KI and KIO_3) or selenium application (Na_2SeO_4 and Na_2SeO_3), soil level of selenium and iodine, respectively, was similar to the geochemical background represented by the control.

Significantly more iodine was determined in soil fertilized with KI than KIO_3 (means from three years of the study − tab. 4). This relation was not however noted in 2014 (fig. 4 A). No significant differences between combinations treated with iodine (both KI and KIO_3) and Na_2SeO_4 were found with respect to iodine concentration in soil. In the case of simultaneous application of iodine with Na_2SeO_3, diverse quantitative relations concerning iodine level were observed in each year of the study.

Table 3. Content of phenolic compounds, oxalates and citrates in lettuce – means from 2012–2014

Treatment (n = 12, ±s.e.)	Phenolic compounds ($mg \cdot 100g^{-1}$ f.w.)	Oxalates ($g \cdot kg^{-1}$ d.w.)	Citrates ($g \cdot kg^{-1}$ d.w.)
Control	45.9 ±4.7^{b}	1.10 ±0.16^{bc}	9.42 ±0.29^{c}
KI	50.2 ±5.9^{c}	1.27 ±0.21^{d}	10.33 ±0.67^{c}
KIO_3	47.2 ±4.8^{bc}	1.00 ±0.12^{abc}	8.03 ±0.95^{b}
Na_2SeO_4	56.3 ±3.1^{de}	0.98 ±0.11^{abc}	10.11 ±0.54^{c}
Na_2SeO_3	38.0 ±4.3^{a}	1.09 ±0.15^{abc}	6.40 ±0.51^{a}
KI + Na_2SeO_4	62.1 ±6.8^{e}	0.94 ±0.10^{a}	9.98 ±1.08^{c}
KIO_3 + Na_2SeO_4	63.3 ±9.2^{e}	0.95 ±0.08^{ab}	9.51 ±0.52^{c}
KI + Na_2SeO_3	49.9 ±7.4^{c}	1.03 ±0.13^{abc}	7.31 ±0.54^{ab}
KIO_3 + Na_2SeO_3	40.1 ±5.3^{a}	1.11 ±0.14^{c}	7.08 ±0.45^{ab}

Means followed by the same letters are not significantly different for $p < 0.05$; s.e. – standard error

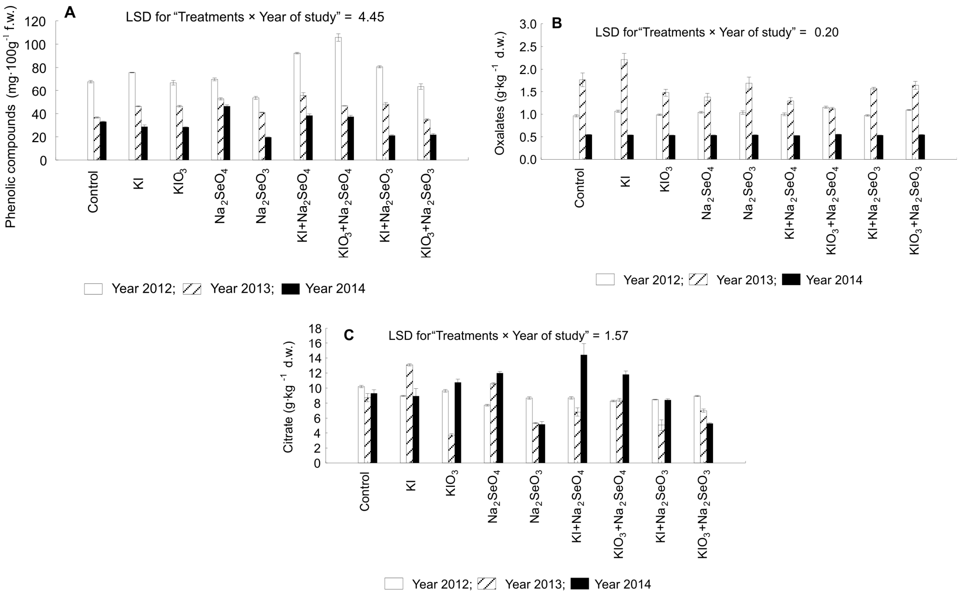

Fig. 3. Content of phenolic compounds (A), oxalates (B) and citrates (C) in lettuce in each year of the study (2012–2014) depending on applied I and Se fertilization. Bars indicate standard error (n = 4)

The highest content of selenium was noted in soil fertilized with KI+Na_2SeO_4 (tab. 4). Interesting is the observation of increased concentration of this element in soil treated with Na_2SeO_4 than Na_2SeO_3 as well as with KI + Na_2SeO_4 over KIO_3 + Na_2SeO_4. It needs to be underlined, however, that obtained relations (tab. 4) were mostly affected by the results for 2012 (fig. 4 A and B) – in the other two years no such relations were observed.

Table 4. Iodine and selenium content in soil after lettuce cultivation – means from 2012–2014

Treatment (n = 12. ±s.e.)	(mg I·kg^{-1} soil)	(mg Se·kg^{-1} soil)
Control	1.54 ±0.06^{a}	1.03 ±0.11^{ab}
KI	6.47 ±0.37^{d}	0.95 ±0.10^{ab}
KIO_3	4.87 ±0.72^{b}	0.71 ±0.04^{a}
Na_2SeO_4	1.31 ±0.14^{a}	1.79 ±0.26^{d}
Na_2SeO_3	0.98 ±0.15^{a}	1.26 ±0.12^{bc}
KI + Na_2SeO_4	5.47 ±0.89^{bc}	2.19 ±0.42^{e}
KIO_3 + Na_2SeO_4	5.33 ±0.93^{bc}	1.21 ±0.07^{bc}
KI + Na_2SeO_3	5.82 ±0.89^{cd}	1.71 ±0.10^{d}
KIO_3 + Na_2SeO_3	5.46 ±1.19^{bc}	1.54 ±0.06^{cd}

Means followed by the same letters are not significantly different for $p < 0.05$; s.e. – standard error

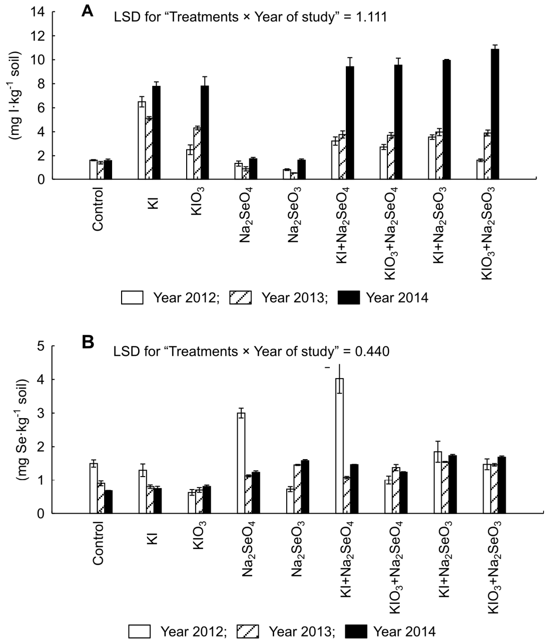

Fig. 4. Iodine (A) and selenium (B) content in soil after lettuce cultivation in each year of the study (2012–2014) depending on applied I and Se fertilization. Bars indicate standard error (n = 4)

DISCUSSION

Iodine and selenium uptake by lettuce plants. Iodides (I^-) are more easily taken up by plants but exhibit higher toxicity than iodate (IO_3^-) ions [Blasco et al. 2010, Kato et al. 2013]. It is assumed that, among selenium compounds, selenates (SeO_4^{2-}) are preferred by plants over selenites (SeO_3^{2-}) [Kopsell and Kopsell 2007]. Selenates or selenites toxicity is influenced by plant type, form of selenium in the growth medium, and the presence of competing ions, such as sulfate and phosphate [Kopsell and Kopsell 2007, Lavu et al. 2013, Li et al. 2015]. Ríos et al. [2008, 2010] revealed greater plant uptake and less toxicity of SeO_4^{2-} rather than SeO_3^{2-} in lettuce cultivation at hydroponic system.

When selenium is applied foliarly, its absorption and accumulation in plants (in both forms: SeO_3^{2-}, SeO_4^{2}) is strictly dependent on weather conditions throughout cultivation, particularly the amount and distribution of rainfall [Poblaciones et al. 2014, Rodrigo et al. 2014]. After soil fertilization, the uptake of all mineral nutrients by roots (including iodine and selenium) is strongly limited by deficient amount of water in soil. Significantly lower accumulation of iodine and selenium (including SeMet and SeCys) in lettuce noted in 2012 was therefore presumably caused by soil drought due to limited rainfall as well as substantially higher air temperature and PAR value.

Table 5. Values of soil-to-plant transfer factor (TF) for iodine and selenium calculated basing on the results of I and Se determination in lettuce using various analytical procedures

Treatments (n = 12, ±s.e.)	TF for iodine in TMAH	TF for iodine in by CVG[1]	TF for selenium in TMAH	TF for selenium in HNO_3
Control	0.80 ±0.04^{a}	0.72 ±0.05^{a}	2.89 ±0.50^{a}	3.88 ±0.97^{ab}
KI	2.15 ±0.19^{c}	1.78 ±0.17^{d}	1.60 ±0.37^{a}	2.12 ±0.16^{a}
KIO_3	1.21 ±0.10^{b}	1.28 ±0.11^{b}	2.61 ±0.63^{a}	3.07 ±0.35^{a}
Na_2SeO_4	0.72 ±0.16^{a}	0.82 ±0.08^{a}	124.22 ±22.04^{f}	92.70 ±22.33^{d}
Na_2SeO_3	0.46 ±0.07^{a}	0.79 ±0.05^{a}	14.94 ±4.23^{c}	12.65 ±3.87^{b}
$KI+Na_2SeO_4$	2.18 ±0.24^{c}	1.44 ±0.09^{bc}	100.70 ±16.70^{d}	72.36 ±12.95^{c}
$KIO_3+Na_2SeO_4$	1.15 ±0.09^{b}	1.33 ±0.11^{bc}	103.21 ±16.53^{d}	75.91 ±13.81^{c}
$KI+Na_2SeO_3$	1.89 ±0.16^{c}	1.50 ±0.06^{c}	13.28 ±3.27^{c}	11.89 ±3.44^{b}
$KIO_3+Na_2SeO_3$	1.24 ±0.11^{b}	1.46 ±0.04^{bc}	9.37 ±2.42^{b}	8.30 ±2.27^{ab}

1 – CVG (cold vapor generation technique). Means followed by the same letters are not significantly different for $p < 0.05$; s.e. – standard error. Iodine and selenium transfer factor [TF] in soil-to-lettuce leaf system was calculated using the following formula: $TF = [C_{\text{plant dry weight}}] / [C_{\text{soil dry weight}}]$, where C was iodine or selenium content in lettuce or soil dry weight

The analysis of the content of discussed elements in lettuce treated only with iodine or selenium confirmed higher absorption (and TF tab. 5) of I^- and SeO_4^{2-} by plants over other tested forms. The higher intake of SeO_4^- form of selenium (confirmed by the TF value) was noted also for its application together with KI and KIO_3. The described relations were observed in each year of the study irrespective of diverse weather conditions and chemical properties of soil. Among tested iodine and selenium compounds applied into the soil, its potentially toxic effects on plants were noted only for Na_2SeO_4 (applied alone or together with KI and KIO_3). In each year of the study, visual symptoms of SeO_4^{2-} toxicity were observed including growth impairment and necrosis of leaf edges. In our opinion the toxic effect of selenate application directly contributed to the decrease in lettuce yield in each year of the study, but to the greatest extent in 2012. It was accompanied by the accumulation of phenolics, intensive synthesis of which is a part of plant response to biotic and abiotic stress, including excessive selenium application [Ríos et al. 2008]. In our study, particularly harmful action of Na_2SeO_4 noted in 2012 was related to the above-mentioned water deficit in soil. As an effect, higher dry matter content (lower leaf hydration) in lettuce was also noted in that year. Despite that, in each year of the study increased accumulation of dry matter was noted in lettuce grown on soil fertilized with Na_2SeO_4 (alone or together with iodine), what was clearly related to yield (biomass) reduction.

Lower efficiency of selenium biofortification of lettuce (lower values of TF – tab. 5), including the level of SeMet and SeCys, after the application of Na_2SeO_3 (as compared to Na_2SeO_4) can be explained by the processes that ions SeO_3^{2-} and SeO_4^{2-} undergo in soil environment. Iron sesquioxides [$Fe(OH)_3$] may cause strong sorption of selenium in soil [Elrashidi et al. 1989, Kopsell and Kopsell 2007]. With deficit amount of water in soil in 2012, the above-mentioned sorption mechanism could have contributed to the lack of selenium enrichment in lettuce fertilized with Na_2SeO_3 – alone or together with KI/KIO_3. Striking is the fact that only in 2012 selenium content in soil after lettuce cultivation was lower after Na_2SeO_3 rather than Na_2SeO_4 application. Conversions of SeO_4^{2-} and SeO_3^{2-} in soil environment occur very slowly [Elrashidi et al. 1989, Kopsell and Kopsell 2007]. During the cultivation of lettuce, which is a fast growing crop, such processes have occurred rather slowly and to a little extent, therefore not significantly affecting plant preference toward selenate or selenite uptake. This assumption seems to be substantiated by the fact that the half of selenium dose was introduced as a top-dressing fertilization. Ríos et al. [2008] found an increased content of Cys and total SH groups in lettuce leaves after the application of SeO_4^{2-} rather than SeO_3^{2-}. Hawrylak and Szymańska [2004] noted the accumulation of non-protein SH groups (characteristic for cysteine) in spinach and tomato roots after SeO_3^{2-} application while SeO_4^{2-} increased its content in shoots. When taken up, SeO_3^{2-} ions are transformed in roots into seleno organic compounds and in such form are then transported to leaves. SeO_4^{2-}, however, is mainly distributed to leaves by xylem and the formation of organic compounds with SeO_4^{2-} after its previous reduction to SeO_3^{2-} in roots is less significant [Zhu et al. 2009]. It can be therefore assumed that in 2012 SeO_3^{-} ions were taken up, efficiently metabolized and accumulated in roots. Due to insufficient soil humidity the transport of seleno organic compounds to leaves was limited when Na_2SeO_3 was introduced into the soil. In our opinion it could have also been the cause of obtaining no effect of selenium biofortification (regarding total selenium or SeMet and SeCys content) in lettuce from the combination with Na_2SeO_3 application (alone or together with KI/KIO_3) cultivated in 2012.

Iodine and selenium interaction. Zhu et al. [2004] revealed no impact of IO_3^- application on selenium content as well as of SeO_4^{2-} treatment on iodine accumulation in spinach. Also Smoleń et al. [2014] did not note any effect of IO_3^- or SeO_4^{2-} present in the nutrient solution in the concentration of 1.0 mg $I \cdot dm^{-3}$ and 0.5 mg Se on root uptake and distribution of selenium and iodine to lettuce leaves.

Iodine uptake after the application of KI and KIO_3 with selenium compounds. Basing on the results from 2013 and 2014, it seems substantiated to state that for the optimal soil humidity, simultaneous fertilization with KI and Na_2SeO_4/Na_2SeO_3

(as compared to KI alone) weakens iodine uptake and accumulation in lettuce leaves. On the other hand, in the conditions of deficit amount of rainfall in 2012 combined with high air temperature (leading to soil drought) no antagonistic influence of SeO_4^{2-} and SeO_3^{2-} was noted with respect to iodine uptake when applied as KI. Despite that, we assume that simultaneous application of KI with selenium compounds (SeO_4^{2-} and SeO_3^{2-}) may limit I^- uptake by plants. It may be the consequence of stronger sorption of iodides in soil when introduced together with Na_2SeO_4 or Na_2SeO_3 than when applied alone. This is further supported by the observation of lower content of available iodine for plants in soil fertilized with KI + Na_2SeO_4 and KI + Na_2SeO_3 than KI alone.

In the case of KIO_3 application of KIO_3, no negative effect of selenium on iodine uptake and accumulation in lettuce was noted. This was particularly valid in the conditions of sufficient water supply in 2013 and 2014.

Selenium uptake and the content of SeMet and SeCys after the application of Na_2SeO_4 combined with KI and KIO_3. In each year of the study, diverse relations concerning total selenium content as well as SeMet and SeCys was noted in lettuce and soil from the combinations with Na_2SeO_4, KI + Na_2SeO_4 and KIO_3 + Na_2SeO_4 application. Therefore, antagonistic effect of iodine on SeO_4^{2-} uptake cannot be named a rule as it could have been concluded from the average results from the three years of the study. This relation was strongly dependent on physicochemical properties of soil and weather conditions throughout lettuce cultivation.

Selenium uptake and the content of SeMet and SeCys after fertilization with Na_2SeO_3 as combined with KI and KIO_3. Irrespective of weather conditions in individual years of the study, a negative influence of simultaneous application of KIO_3 + Na_2SeO_3 (as compared to Na_2SeO_3 alone) on selenium uptake and accumulation as well as SeMet content in lettuce was revealed. It was not however reflected by selenium content in soil after lettuce cultivation. Such effect can be explained basing on the hypothesis of IO_3^- reduction to I^- that occurs in roots after its uptake or the incorporation of formed iodides into organic compounds within the root system [Cseh and Böszörményi 1964]. Iodate ions can also be reduced by soil microorganisms into I_2 or I^- forming on root surface [Kato et al. 2013] directly affecting the redox potential value within the rhizosphere. Therefore, additional expense of energy for IO_3^- reduction in roots may have interfered with SeO_3^{2-} uptake from soil or its transformation into organic forms of Se (including SeMet and SeCys) in roots. The final consequence could be the limited distribution of organic Se into lettuce leaves.

In each year of the study in plants treated with of KIO_3 + Na_2SeO_3 a significant decrease in the content of phenolic compounds and citrates was noted as compared to the control. This could have been the cause of lower concentration of citrates in lettuce – one of the key compounds in the Krebs cycle – and limited biosynthesis of secondary metabolites, including SeMet and phenolic compounds. Such negative relations were noted in the years with higher amount of rainfall (2013–2014, tab. 3 and fig. 3). There also may be the direct consequence of higher selenium uptake by plants in these years than in 2012. However, no decrease in plant biomass was noted in plants from this combination what substantiates the conclusion that simultaneous application of KIO_3 + Na_2SeO_3 in tested doses did not exceed tolerance levels of lettuce towards both ions (IO_3^- and SeO_3^{2-}).

The assessment of analytical methods of iodine and selenium determination in lettuce. Basically most of the studies on plant biofortification document the analysis of iodine [Voogt and Jackson 2010, Voogt et al. 2010, Blasco et al. 2010, Kato et al. 2013] or selenium [Ríos et al. 2008, 2010, Poblaciones et al. 2014] content in plants using only one technique.

Numerous procedures differing with respect to applied mineral acids (HNO_3, HCl, $HClO_4$, H_2SO_4) and/or its mixtures as well as temperature and pressure parameters during digestion have been developed in order to minimize the losses of selenium and other mineral nutrients [Bañuelosa and Akohouea 1994, Zhou et al. 1997, Pasławski and Migaszewski 2006]. In the case of iodine, it is volatilized in the form of I_2 under acidic conditions yet the addition of $AgNO_3$ stabilizes iodine when samples are digested in HNO_3 [Varga 2007]. In our studies, lower selenium contents (as well as TF values) were noted after sample digestion in HNO_3 than after using TMAH what indicates higher Se losses when applying the former method. When analyzing iodine and selenium content after TMAH extraction, a complex matrix effect must be however taken into account. A thick, dark-brown consistency of such analyzed samples hardens aerosol formation in cyclonic spray chamber. Studies conducted by Smoleń et al. [2011] revealed that the values of correlation coefficients for iodine content in spinach after sample incubation with TMAH as compared to 2% CH_3COOH extraction depended not only on applied matrix but also iodine dose, method of its application (soil fertilization and/or foliar spraying) and the type of cultivation – field or pot experiment.

The applied procedure of iodine analysis using CVG is preceded by mineral digestion, not extraction as for iodine analysis in TMAH. Significantly, Vtorushina et al. [2009] – the authors of that method – proposed the optimal HNO_3:$HClO_4$ volume ratio to determine iodine content from its trace values to the level of approximately 150 mg $I \cdot kg^{-1}$ d.w.

The aim of iodine and selenium biofortification from the consumer's point of view. For the consumer, it is important that selenium occurs in the food (including vegetables) in its organic forms: SeMet, SeCys, MeSeCys as they exhibit the most positive effect on its organism [Pyrzynska 2009]. In our studies, lettuce contained selenium mostly in its organic forms. However, simultaneous application of KI/KIO_3 with Na_2SeO_3 strongly limited the percentage share of SeMet and SeCys in the total selenium content in plants. Positively, the presence of SeO_4^{2-} and SeO_3^{2-} – harmful even at low concentrations – were not detected in lettuce. The lack of free iodine ions in lettuce suggested the occurrence of this element in its organic compounds, which availability to human organism from biofortified vegetables is unlimited [Tonacchera et al. 2013].

Daily requirements of iodine and selenium (RDA) for adults is 150 µg I and 55 µg Se, respectively, while for pregnant and nursing women – 200–300 µg I and 60–70 µg Se [Food and Nutrition Board 2000, Andersson et al. 2007]. The optimal iodine to selenium weight ratio in human diet is therefore within the range of 2.7−5:1 (the optimum ratio of molar mass of both elements is within 4.4–8.8:1) and can be proposed as a necessary parameter describing the efficiency of simultaneous biofortification of plants with these elements.

The average serving of leafy vegetables is approximately 50 g f.w. [Voogt et al., 2010]. Taking this into account, it can be estimated that lettuce could be considered as a good source of iodine and selenium for consumer (tab. 6). In further studies, however, appropriate I and Se doses must be established in order to supply the better coverage of RDA. In our study, close to the optimal I:Se weight ratio was obtained after the fertilization with KI only, with the values of 2.84−5.34:1, depending on the applied analytical method.

Table 6. Iodine and selenium content in 50g portion of fresh lettuce leaves including the percentage coverage of daily requirement for I and Se as well as I:Se ratio in lettuce calculated basing on the results of I and Se determination using various analytical procedures – means from 2012–2014

Treatments (n = 12)	I and Se content in 50 g portion of fresh lettuce leaves (µg)		Coverage of daily requirement for I and Se by 50g portion of fresh lettuce leaves (%)		I : Se ratio in lettuce	
	iodine (TMAH /CVG[1])	selenium (TMAH /HNO_3)	iodine (TMAH /CVG)	selenium (TMAH/HNO_3)	I : Se (both in TMAH)	I by CVG : Se in HNO_3
Control	3.3/2.9	6.7/9.6	2.2/1.9	12.2/17.4	0.49 : 1	0.30 : 1
KI	14.1/11.8	2.6/4.1	9.4/7.8	4.8/7.5	5.34 : 1	2.84 : 1
KIO_3	8.2/8.5	4.3/5.9	5.5/5.7	7.8/10.8	1.92 : 1	1.43 : 1
Na_2SeO_4	3.0/4.1	328.2/246.4	2.0/2.7	596.8/448.0	0.01 : 1	0.02 : 1
Na_2SeO_3	1.9/3.5	30.4/27.1	1.3/2.3	55.3/49.3	0.06 : 1	0.13 : 1
KI+Na_2SeO_4	13.2/11.2	268.0/186.6	12.5/7.5	487.3/339.2	0.05 : 1	0.06 : 1
KIO_3+Na_2SeO_4	9.3/10.3	274.3/200.1	6.2/6.9	498.7/363.7	0.03 : 1	0.05 : 1
KI+Na_2SeO_3	12.4/11.1	28.7/26.9	9.9/7.4	52.2/48.8	0.43 : 1	0.41 : 1
KIO_3+Na_2SeO_3	8.2/10.2	20.5/19.4	5.5/6.8	37.2/35.3	0.40 : 1	0.52 : 1

1 – CVG (cold vapor generation technique)

At the same time, lettuce from this combination would cover adult's requirements for iodine and selenium in 7.8–9.4 and 4.8–7.5%, respectively. Due to much easier uptake of selenium over iodine by plants, simultaneous application of these elements led to unfavorable ratio of its content in lettuce, particularly for Na_2SeO_4 application (rather than Na_2SeO_3). In combinations with Na_2SeO_3 (applied alone or together with KI/KIO_3) 50 g of lettuce would cover 35.3–55.3% of adult's daily requirements for selenium. For Na_2SeO_4 application selenium content absorbed from 50 g portion of lettuce would substantially exceed the recommended level.

It is worth noting that we recorded improved iodine assimilability and its metabolism in rats fed with lettuce biofortified with iodine (KI) in relation to lettuce not biofortified with iodine or a diet containing synthetic KI [Kopeć et al. 2015]. Furthermore, we demonstrated the inhibition of Caco-2 cancer cell proliferation after treatment with iodine-biofortified lettuce (BFL) extract, but not potassium iodide (KI), and BFL-mediated induction of mitochondrial apoptosis and/or cell differentiation. This study showed 1326 differently expressed Caco-2 transcripts after treatment of iodine-biofortified (BFL) and non-fortified with iodine lettuce extract [Koronowicz et al. 2016].

CONCLUSION

Simultaneous application of iodine and selenium (in all tested chemical forms) limited selenium accumulation and the content of SeMet and SeCys. Particularly unfavorable, with respect to lettuce quality (but not yield), was soil fertilization with KIO_3 + Na_2SeO_3. Despite the decrease in selenium content, lower amounts of SeMet was accumulated in lettuce in the years with greater amount of rainfall (2013–2014) than with water deficit (2012).

Presented relations of I:Se weight ratios and the coverage of adult's requirements towards both elements by 50 g portion of lettuce indicate the need to conduct further studies on the development of agrotechnical methods of double biofortification of plants with iodine and selenium. Such methods should also improve iodine mobility and soil-to-plant transfer. It seems advisable to determine the suitable dosage of selenium in the form of selenites and selenates as an approximate ten-fold difference in the effect of selenium biofortification of lettuce was noted. These solutions should lead to obtaining sustainable I:Se weight ratio in the yield within the range of (2.84−5.34):1 (molar mass ratio (4.4–8.8):1). In our opinion, in soils rich in selenium additional fertilization with this element should be neglected as to avoid the risk of obtaining yield with its excessive content, potentially harmful for the consumer. Additionally a disturbance in the optimal I:Se ratio in yield may be then noted even after applying relatively high iodine doses.

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BIOFORTYFIKACJA SAŁATY (*Lactuca sativa* L.) W JOD I SELEN PRZY NAWOŻENIU DOGLEBOWYM RÓŻNYMI FORMAMI CHEMICZNYMI TYCH PIERWIASTKÓW

Streszczenie. Stosunkowo niewiele wiadomo na temat interakcji pomiędzy jodem i selenem w roślinach. Stanowi to problem w opracowaniu racjonalnych agrotechnicznych zasad biofortyfikacji roślin w te składniki. Celem badań było określenie wpływu nawożenia doglebowego różnymi formami jodu (I^- i IO_3^-) i selenu (SeO_3^{2-} i SeO_4^{2-}) na plon, wydajność biofortyfikacji oraz wybrane chemiczne właściwości roślin sałaty. Badania (przeprowadzone w latach 2012–2014), obejmowały następujące kombinacje nawożenia doglebowego sałaty ‘Valeska’: kontrola (bez nawożenia jodem i selenem), KI, KIO_3, Na_2SeO_4, Na_2SeO_3, KI + Na_2SeO_4, KIO_3 + Na_2SeO_4, KI + Na_2SeO_3, KIO_3 + Na_2SeO_3. Związki jodu i selenu były aplikowane dwukrotnie: przedsiewnie oraz pogłównie (każda aplikacja po 2,5 kg $I \cdot ha^{-1}$ + 0,5 kg $Se \cdot ha^{-1}$) – całkowita zastosowana dawka wynosiła 5 kg $I \cdot ha^{-1}$ oraz 1 kg $Se \cdot ha^{-1}$. Jedynie nawożenie Na_2SeO_4 (osobno i łącznie z KI i KIO_3) wywierało silnie toksyczny wpływ na rośliny. Towarzyszyło temu największe nagromadzenie selenu oraz selenometioniny (SeMet) i selenocysteiny (SeCys) w sałacie. Akumulacja I i Se w sałacie była odpowiednio wyższa po nawożeniu KI niż KIO_3 oraz Na_2SeO_4 niż Na_2SeO_3. Równoczesne nawożenie jodem i selenem powodowało zmniejszenie zawartości selenu, SeMet i SeCys w sałacie – zwłaszcza po nawożeniu KIO_3 + Na_2SeO_3. Równoczesne nawożenie KI z obiema formami selenu, w porównaniu z aplikowaniem samego KI, obniżało zawartość jodu w sałacie. W przypadku obiektów KIO_3, KIO_3 + Na_2SeO_4 i KIO_3 + Na_2SeO_3 stwierdzono porównywalny między nimi stopień akumulacji jodu w sałacie.

Słowa kluczowe: biofortyfikacja, jakość biologiczna, selenometionina, selenocysteina, generacja zimnych par jodu

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