Polish J. of Environ. Stud. Vol. 16, No. 1 (2007), 57-62

Original Research

Changes in the Contents of Some Carbohydrates in Vegetables Cumulating Lead

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Received: October 25, 2005
Accepted: August 4, 2006

Abstract

Six vegetable crop species were investigated: lettuce, spinach, radish, carrot, red beet and onion. Each species was represented by two cultivars. The vegetables were cultivated in pots on substrates containing 0, 250 or 500 mg·kg^{-1} d. m. of lead. Increasing lead doses caused a decrease in sucrose content in edible plant parts accompanied by an increase in starch content in comparison with vegetables cultivated on comparative substrate. No constant tendency of changes in glucose and fructose levels in the studied plant tissues were registered under the influence of cumulated lead. In the case of root vegetables and onion, in which sucrose is the dominating soluble sugar (approximately 80% of total soluble sugars), a weakening of sweet taste may be expected if the plants are cultivated in conditions of lead pollution.

Keywords: carrot, fructose, glucose, lead cumulation, lettuce, onion, radish, red beet, spinach, starch, sucrose

Introduction

Lead (Pb), which is widely spread throughout the environment, reveals relatively high reactivity and strongly affects many physiological processes. It is considered the most phytotoxic among heavy metals [1]. Research on lead's effects on the structure of photosynthetic apparatus and the course of photosynthesis point to numerous disturbances caused by this metal interference [2]. Changes in many products of photosynthesis, particularly carbohydrates, are observed in tissues under the influence of lead. Changes in total soluble sugars in tissues of plants grown in environments polluted with this metal cannot be determined unanimously. A decrease in their level was detected in *Chlorella vulgaris* colonies [4] and *Salvinia natans* [5], in rice plants [6], lemon grass [7], safflower [8] and in soybean verrucas [9]. An increase in total sugars in similar conditions was also observed in tomato plants [10], in carrot roots [11], in seedlings of rice [12] and sesame [13] and in *Lemma gibba* cultures [14]. Results of the author's earlier papers [16, 17] also allow us to assume that total change in soluble sugars in plants occurring under the influence of absorbed lead is difficult to predict because of the fact that the term "soluble sugars" comprises mainly various mono and disaccharides occurring in different amounts in cell sap. The destructive effect of lead was also proved in the case of polysaccharide biosynthesis. A negative influence of this metal on starch metabolism in germinating pea seeds was discussed by Vikas et al. [18]. Changes in the course of structural carbohydrate synthesis were also revealed, such as inhibition of cellulose synthesis and activation of hemicellulose in wheat seedlings cumulating lead [19].

Research results obtained so far have shown that carbohydrate metabolism is impaired by heavy metals in various ways. These changes may, to a considerable extent, be a result of the inactivation of enzymes participating in their synthesis or hydrolysis. It is a known fact that heavy metals change activity of invertase and amylases [20, 18].

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Enzymes contained in their active centres SH functional groups of cysteine should be particularly prone to lead effect, because this group reveals a strong affinity to lead. An example of such an enzyme is β-amylase.

The work presents results of research on changes in the content of the most commonly occurring soluble sugars: glucose, fructose and sucrose in selected vegetables grown on substrates containing lead. Changes of starch content, i.e. the most common reserve sugar, were also discussed.

Experimental Procedures

This pot experiment was established in 2002 and 2003 in the last decade of April on the Experimental Farm of the Faculty of Horticulture, Agricultural University of Kraków. Six species of popular vegetables were investigated, each of which was represented by two cultivars. These included: lettuce (*Lactuca sativa* L.), Sonata and Marysieńka cvs., spinach (*Spinacia oleracea* L.) Matador and Asta F_1 cvs., radish (*Raphanus sativus* L. subvar. *radicula* Pers.) Saxa and Carmen cvs., red beet (*Beta vulgaris* L.), Egipski and Glob F_1cvs., carrot (*Daucus carota* L.), Kamila F_1 and Kalina F_1 cvs., onion (*Allium cepa* L. var. *cepa* Helm), Rawska and Efekt cvs. The substrate consisted of crushed high peat and washed river sand. Both components were characterized by low concentrations of lead (10 mg·kg^{-1} d.m.), below the upper limit of natural background of this metal registered in Polish soils as 18 mg·kg^{-1} d.m. Macroelements: 250 mg N, 250 mg K, 100 mg P and 60 mg Mg per a kilogram of dry mass were supplied into the substrates as pure reagent salts. Microelements were added using the microelement part of a multi component MIS-4 fertilizer in the amount recommended by the producer. The acidity of such a prepared substratum was lowered to pH 6.5 using chalk. Mitscherlich pots, 5 dm^3 in volume were filled with the substrate and simultaneously lead was added as lead acetate solution. Each cultivar was cultivated on three substrates with different levels of lead pollution: 0, 250 and 500 mg Pb·kg^{-1} d.m., 8 pots per substrate. The pots were placed in the hotbed and seeds of spinach, radish, red beets and carrot were sown into them and 4-week-old seedlings of lettuce and onion were planted. The plants were watered with distilled water within 70% of capillary water capacity. After the appearance of the first pair of leaves, eight equal plants were left per pot. Lettuce was an exception, as it was planted three pieces per pot. The vegetables were gathered at their harvest maturity, thoroughly washed in running water and then 25 plants from each object were selected for analyses (lettuce sample was 15 heads). Edible plant parts, which in individual species were leaves, roots or bulbs, were analyzed.

The content of glucose, fructose and sucrose was determined by the ferrocyanide method [21]. Starch was assessed by colorimeter using iodine solution in potassium iodide [22]. Total lead concentrations in all the crop species were essayed by atomic absorption [23] by Varian SpectrAA-20 spectrophotometer in the acetylene-air flame after material incineration at 450^{o}C and solving the remains in 10% nitric acid. The results were elaborated statistically by ANOVA in the completely randomized design using t-Student test at the significance level 0.05. Because of repeating tendencies in individual years and to simplify the presentation results, two-year means were given in the Tables.

Table 1. Lead content in edible parts of vegetables cultivated on substrate with diversified Pb level (mg·kg^{-1} f. m.).

Pb dose (mg·kg^{-1} d. m. substrate)	Lettuce	Spinach	Radish	Onion	Red Beet	Carrot
	Sonata	Asta F_1	Carmen	Efekt	Glob F_1	Kalina F_1
0	0.33a*	0.59a	0.57a	0.55a	1.05a	0.84a
250	1.05c	1.70c	1.34b	0.83bc	3.30c	3.70b
500	1.30d	2.24d	2.19c	0.94bc	4.58d	6.07d
	Marysieńka	Matador	Saxa	Rawska	Egipski	Kamila F_1
0	0.86b	1.26b	0.57a	0.44a	2.88b	0.71a
250	1.43e	2.32d	1.81bc	0.68ab	5.77e	3.33b
500	1.63f	3.37e	3.36d	1.10c	6.75f	5.08c
	Mean for species					
0	0.59a	0.92a	0.57a	0.50a	1.96a	0.78a
250	1.24b	2.01b	1.57b	0.76b	4.53b	3.51b
500	1.47c	2.80c	2.78c	1.02c	5.66c	5.57c

* Values marked with the same letter within the same species and means for species do not differ significantly.

Results

Increased lead doses in the substrate caused an increase in its concentrations in all tested vegetable species (Table 1). Mean Pb content in lettuce leaves cultivated on the comparative substrate was 0.59 mg·kg^{-1} f.m. Under the influence of 250 mg Pb·kg^{-1} d.m. dose it grew by ca. 110%, while in effect of applied 500 mg Pb·kg^{-1} d.m. by ca. 149% of the initial content. The selected lettuce cultivars differed with their lead cumulation, Sonata cv. accumulated on average by 32% less than Marysieńka cv. Mean lead content in spinach leaves gathered from the substratum receiving 0 mg Pb·kg^{-1} d.m. was 0.92 mg Pb·kg^{-1} f.m. and increased by ca. 118% and ca. 204% after the application of subsequent lead doses. Asta F_1 and Matador differed significantly with respect to Pb cumulation, the first accumulated by 35% more lead than the other one. In radish roots from the substrate free of lead about 0.57 mg of this metal was assessed per 1 kg^{-1} f.m. Supply of subsequent Pb doses to the substrate resulted in its elevated concentrations in radish by ca.175% and by ca. 388% in comparison with the control material. Lead content in Carmen roots was by 29% lower than this metal amount detected in Saxa roots. Onion growing on substrate unpolluted by lead contained on average 0.50 mg Pb·kg^{-1} f.m. When a dose of 250 mg Pb·kg^{-1} d.m. was supplied, this metal concentration in onion increased by ca. 52% and by 104% when the dose was doubled. No differentiation in lead cumulation between cultivars was registered for Efekt and Rawska cultivars. In red beetroots cultivated on comparative substrate on average 1.96 mg Pb·kg^{-1} f.m. was assessed. A supplement of 250 and 500 mg lead·kg^{-1} d.m. resulted in the increase in its root concentrations by about 131 and 189% in comparison with beetroots from the substrate free of lead. Glob F_1 beetroots cumulated by 42% less lead than beetroots Egipski cv. Mean Pb concentration of 0.78 mg·kg^{-1} f.m. was noted in carrot roots harvested from the control substrate. After adding subsequent doses of lead to the substrate the level of this element in carrot raised by ca. 350% and 614% in comparison with the roots from substrate with zero dose. Kamila F_1 carrot had by 14% less Pb than Kalina F_1 cultivar.

Changes in glucose concentrations in the edible parts of vegetables cultivated on substrates with increasing lead contents are presented in Table 2. The level of this sugar declined on average for species in lettuce leaves, radish roots and beetroots respectively by 12.5%, 9.5% and 40.0% – after increasing lead content in the substrate from 0 to 500 mg Pb·kg^{-1} d.m. At the same time in the same conditions glucose concentration increased subsequently by 11.8%, 7.1% and 20.3% in spinach leaves, onion and carrot roots. Within the respective species only an analysis of both red beet cultivars and both carrot cultivars revealed the same tendencies of changes in the content of discussed components under the influence of cumulated lead. Changes of glucose levels registered for the other species are resultants for the tested cultivars.

Fructose concentrations in vegetables grown on substrates with diversified Pb content were given in Table 3. After increasing Pb content in the substrate from 0 to 500 mg·kg^{-1} d.m. mean content of this sugar grew in lettuce leaves by 5.2%, in onion by 8.4% and in beetroots by 36.9%, whereas it decreased by 19.5% in spinach leaves and by 17.0% in carrot roots, but did not change significantly in radish roots. Fructose content in tissues of culti-

Table 2. Glucose content in edible parts of vegetables cultivated on substrate with diversified Pb level (mg·kg^{-1} f. m.).

Pb dose (mg·kg^{-1} d. m. substrate)	Lettuce	Spinach	Radish	Onion	Red Beet	Carrot
	Sonata	Asta F_1	Carmen	Efekt	Glob F_1	Kalina F_1
0	37.07 c*	9.60 a	12.33 c	13.33 e	11.47 c	13.93 a
250	33.20 b	10.47 a	10.47 b	12.27 d	10.87 c	15.53 b
500	29.47 a	12.87 b	9.83 b	12.70 b	8.73 b	17.37 c
	Marysieńka	Matador	Saxa	Rawska	Egipski	Kamila F_1
0	37.93 c	15.80 c	7.67 a	5.90 a	8.60 b	14.10 a
250	35.80 c	15.83 c	7.97 a	6.53 b	5.47 a	15.43 b
500	36.20 c	15.53 c	8.27 a	7.90 c	4.90 a	16.37 bc
	Mean for species					
0	37.50 b	12.70 a	10.00 b	9.62 a	10.03 c	14.02 a
250	34.50 a	13.15 a	9.22 a	9.40 a	8.17 b	15.48 b
500	32.83 a	14.20 b	9.05 a	10.30 b	6.82 a	16.87 c

* Values marked with the same letter within the same species and means for species do not differ significantly

vars from the same species was changing diversely under the influence of accumulated lead and the same tendency for changes was noticed only for spinach.

Changes of sucrose contents in the edible parts of vegetables cultivated on the substrate with increasing lead doses were presented in Table 4. Mean content of this sugar calculated for a species was decreasing with increasing substrate lead concentrations. It decreased under the influence of 250 mg·kg^{-1} d.m. and then 500 mg·kg^{-1} d.m. in spinach leaves by 12.6% and 26.8%, in radish roots by 23.0% and 36.8%, in onion by 8.5% (because of the higher dose), in beetroots by 9.4% and 15.6% and in carrot roots by 27.9% and 45.1%. Only in lettuce was no diversification in sucrose concentration registered in plants under the influence of growing Pb doses. The analysis of individual cultivars revealed a decline in sucrose level in effect of accumulated lead and only in three cases were no significant changes of its content noted.

Table 5 shows starch content in vegetables cultivated on the substrates with increasing lead doses. Its mean content was increasing with growing Pb dose in the substrate. A supplement of 250 mg lead·kg^{-1} d.m. and then 500 mg·kg^{-1} d.m. caused an increase in starch concentrations in lettuce leaves by 11.0% and 14.0%, in spinach leaves by 6.9% and 16.2%, in radish roots both doses on average by 9.2%, in onion respectively by 10.8% and 45.9%, in beetroots both doses on average by 9.9%,in carrot roots by 19.4% and by 48.2%. Changes in starch concentrations in tissues of individual cultivars also revealed a growing character except of this component content in Saxa radish roots and in Rawska onions, where no statistically significant differences were assessed.

Discussion of Results

Analyses conducted on two monosaccharides: glucose and fructose in six vegetable species cumulating lead did not produce results which would allow for drawing general conclusions concerning the direction of changes of this sugars contents under the influence of lead. Even in the case of species in which the same organ is an edible art (lettuce and spinach, beetroots and carrots) no uniform tendency could be found. Reports of similar investigations that can be found in literature are also diversified. For example an increase in glucose level was observed in pea seedlings growing on a substrate containing lead [24], whereas in birch leaves and spruce and pine needles subjected to lead dust emission a decline was registered in this carbohydrate content [25].

Formulating conclusions common for the whole research material was possible for sucrose and starch. Subsequent doses of Pb 250 and 500 mg·kg^{-1} d.m. supplied to the substrate caused a decrease in sucrose in the tissues of investigated vegetables even by 45% (carrot, a dose of 500 mg Pb·kg^{-1} d.m.). The work by Poskuta et al. [24] quoted above reports that an increase in Pb concentration in the medium from 0 to 800 mg·dm^3 caused a decline in sucrose concentrations in pea plants by approximately 40%. Similar observations were reported by Selim [11].

In the discussed experiment the maximum 46% increase in starch concentration in vegetable organs under the influence of diversified lead doses was obtained in onions at a higher Pb dose. The other authors [14, 15]

Table 3. Fructose content in edible parts of vegetables cultivated on substrate with diversified Pb levels (mg·kg^{-1} f. m.).

Pb dose (mg·kg^{-1} d. m. substrate)	Lettuce	Spinach	Radish	Onion	Red Beet	Carrot
	Sonata	Asta F_1	Carmen	Efekt	Glob F_1	Kalina F_1
0	72.80 a*	24.03 d	14.50 c	11.67 c	3.80 a	8.60 d
250	72.13 a	22.57 c	14.03 bc	12.07 c	4.23 a	5.93 b
500	78.27 b	18.20 a	13.73 b	10.43 b	3.87 a	4.67 a
	Marysieńka	Matador	Saxa	Rawska	Egipski	Kamila F_1
0	69.63 a	20.83 b	12.90 a	8.53 a	3.83 a	7.83 c
250	70.27 a	18.93 a	13.97 bc	8.90 a	5.63 b	7.97 c
500	71.60 a	17.90 a	14.10 bc	11.47 c	6.60 c	8.97 d
	Mean for species					
0	71.22 a	22.43 c	13.70 a	10.10 a	3.82 a	8.22 b
250	71.20 a	20.75 b	14.00 a	10.48 ab	4.93 b	6.96 a
500	74.93 b	18.05 a	13.92 a	10.95 b	5.23 b	6.82 a

* Values marked with the same letter within the same species and means for species do not differ significantly

Table 4. Sucrose content in edible parts of vegetables cultivated on substrate with diversified Pb levels (mg·kg⁻¹ f. m.).

Pb dose (mg·kg^{-1} d. m. substrate)	Lettuce	Spinach	Radish	Onion	Red Beet	Carrot
	Sonata	Asta F_1	Carmen	Efekt	Glob F_1	Kalina F_1
0	16.87 a*	11.70 ab	22.23 d	63.00 a	53.80 c	53.33 e
250	17.43 a	11.37 ab	18.57 c	62.50 a	52.90 bc	46.87 d
500	17.00 a	11.93 ab	16.63 b	62.93 a	51.63 b	32.23 c
	Marysieńka	Matador	Saxa	Rawska	Egipski	Kamila F_1
0	28.33 c	16.53 c	29.93 e	74.47 c	61.30 d	47.93 d
250	27.17 b	12.73 b	16.97 b	72.33 b	51.43 b	26.17 b
500	27.20 b	8.73 a	12.53 a	62.90 a	45.53 a	23.40 a
	Mean for species					
0	22.60 a	14.12 b	23.08 c	68.73 b	57.55 c	50.63 c
250	22.30 a	12.0 ab	17.77 b	67.42 b	52.17 b	36.52 b
500	22.10 a	10.33 a	14.58 a	62.92 a	48.58 a	27.82 a

* Values marked with the same letter within the same species and means for species do not differ significantly

Table 5. Starch content in edible parts of vegetables cultivated on substrate with diversified Pb levels (mg·kg^{-1} f. m.).

Pb dose (mg·kg^{-1} d. m. substrate)	Lettuce	Spinach	Radish	Onion	Red Beet	Carrot
	Sonata	Asta F_1	Carmen	Efekt	Glob F_1	Kalina F_1
0	185.73 c*	113.93 a	56.93 c	58.27 b	58.27 c	92.40 b
250	212.13 e	128.40 b	62.87 d	65.87 c	65.87 d	117.07 c
500	215.93 e	148 40 d	66.53 e	104.27 d	66.40 d	137.07 e
	Marysieńka	Matador	Saxa	Rawska	Egipski	Kamila F_1
0	167.00 a	139.47 c	35.67 ab	48.40 a	50.53 a	84.27 a
250	179.27 b	142.40 c	38.00 b	52.33 ab	52.77 b	93.87 b
500	194.40 d	146.00 d	34.80 a	51.37 a	54.13 b	124.67 d
	Mean for species					
0	176.37 a	126.70 a	46.30 a	53.33 a	54.40 a	88.33 a
250	195.70 b	135 40 b	50.43 b	59.10 b	59.32 b	105 47 b
500	202.67 c	147.20 c	50.67 b	77.82 c	60.27 b	130.87 c

* Values marked with the same letter within the same species and means for species do not differ significantly

reported a decrease in starch content in tissues of plants grown on substrates containing lead. However, Balsberg-Pahlson [25] reported that starch content in leaves and needles of trees within the lead emission elevated by between 25 and 70% in comparison with trees from outside the emission.

The experiment conducted demonstrated that the biological value of vegetables cultivated in conditions of lead pollution is significantly affected by changes of the studied carbohydrates: glucose, fructose, sucrose and starch concentrations. In the case of root crops where sucrose is the dominating sugar (constituting approximately 80% of the analyzed sugars in beetroots and onion) a weakening of their characteristic sweet taste may be expected, whereas the same effect may not be observed in leaf vegetables.

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