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Maria GAWĘDA[1] and Zofia NIZIOŁ-ŁUKASZEWSKA[1]

EFFECT OF LEAD CUMULATION ON BORON CONTENT IN SOME VEGETABLES

WPŁYW KUMULACJI OŁOWIU NA ZAWARTOŚĆ BORU W NIEKTÓRYCH WARZYWACH

Abstract: Six species of vegetable plants: lettuce *Lectuca sativa* L., spinach *Spinacia oleracea* L., radish *Raphanus sativus* L. subvar. *radicula* Pers., onion *Allium cepa* L. var. *cepa,* red beets *Beta vulgaris* L. and carrot *Daucus carota* L. were cultivated in pots on a substrate containing lead dosed 0, 250 and 500 mg · kg^{-1} d.m.

Increase of lead content in substrate caused its intensive cumulating in edible parts of the tested vegetable species. At the same time boron content increased in tissues of all analysed plants: in lettuce leaves by 9.7 % after supplying 250 mg Pb · kg^{-1} d.m., and by 19.8 % after supplying 500 mg Pb · kg^{-1} d.m., in spinach leaves by 13.8 % (only under the influence of higher dose), in radish roots by 5.1 and 24.6 %, in onion by 23.9 and 39.1 %, in beetroots by 14.9 and 41.2 % and in carrot roots by 8.6 and 24.6 %.

Keywords: lead accumulation, boron, lettuce, spinach, radish, onion, red beet, carrot

One of the undesired results of rapidly developing civilisation of the past century has been excessive amount of heavy metals in the environment. Numerous works have focused on accumulation of these metals in various plants, we also know a lot about the possibilities to prevent their intensive accumulation in plant tissues. However, far less may be said about the results of heavy metal toxicity in plants, such as disorders in mineral nutrition or changes in their metabolism. Although external symptoms of heavy metal contamination in plants appear very late, there are proofs that many changes inside their cells occur fast and are far ahead of macroscopic effects.

Changes in mineral uptake are among first plant responses to heavy metal presence in the substrate. These relationships have been relatively best known in case of lead due to heavy environmental pollution with this metal and considerable hazard it poses to humans. Nitrate(V) ion uptake [1] and sulphate ion absorption [2] decrease under the influence of lead. So, it seems that, a strong antagonism between lead and phosphorus is connected with its intense retardation in the substratum through plumbous ions [3]. In

[1] Department of Vegetable Crops and Horticulture Economics, University of Agriculture in Krakow, al. 29 Listopada 54, 31–425 Kraków, Poland, phone: +48 12 662 52 23, email: mgaweda@bratek.ogr.ar.krakow.pl

case of some plants, lead stimulates potassium uptake [4], but in general the effect of outflow of potassium ion has been observed in result of increased cell membrane permeability [5]. There is some information about either increased calcium content in plants grown in lead polluted soils [6] or about decreased content under similar conditions [7]. Similarly, a view upon changes in magnesium content in plant tissues under lead influence is somewhat unclear [8]. Lead is an inhibitor of uptake of many microelements actively occurring as bivalent cations: iron, manganese, zinc, copper and cobalt [3, 9], competing with them for active positions of carriers, enzymes or cell membranes.

Boron is a very important microelement in plant metabolism. The problem of its response to increased lead concentration in plant tissue remains unsolved. The work aimed to determine how boron content changes in edible parts of selected vegetables cultivated on substrates containing elevated lead content.

Materials and methods

A pot experiment was conducted during two growing periods in 2005 and 2006 at a Research Farm of Faculty of Horticulture, University of Agriculture in Krakow. Test materials were provided by six species of popular vegetables, each represented by two randomly chosen cultivar varieties. These comprised: lettuce *Lactuca sativa* L., 'Sonata' and 'Marysienka' 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_1 cvs., carrot *Daucus carota* L., 'Kamila' F_1 and 'Kalina' F_1 cvs., and onion *Allium cepa* L. var. *cepa* Helm., 'Rawska' and 'Efekt' cvs. Substrate was prepared of rubbed highmoor peat and washed river sand. Both components are characterised by a low content of lead (below 10 mg $\cdot$ kg^{-1} d.m.), which places them below the upper limit of this metal natural background in Polish soils determined as 18 mg $\cdot$ kg^{-1} d.m. [10]. Macroelements, dosed 250 mg N, 250 mg K, 100 mg P and 60 mg Mg per a kilogram of dry matter were supplied to the substrate as chemically pure salts. Microelements were added using the microelement part of multielement MIS-4 fertilizer in the amount recommended by the producer. Acidity of the prepared substrate was decreased to pH 6.5 using chalk. Determined boron content in this substrate was ca 0.90 mg $\cdot$ kg^{-1} d.m. The results of basic soil analysis before experiment were given in Table 1.

Table 1

The content of macroelements, pH and salinity of the substrate

NH_4	NO_3	P	K	Ca	Mg	pH	Salinity
[mg $\cdot$ dm^{-3}]							[g NaCl $\cdot$ dm^3]
85.8	49.0	60.8	298.2	1693	164.0	6.83	1.46

The experiment was set up in the last decade of April. Mitscherlich's pots, 5 dm^3 in volume, were filled with substrate and lead added simultaneously in the form of lead acetate. Each variety was cultivated on three substrates to various degrees contaminated with lead: 0, 250 and 500 mg Pb · kg^{-1} d.m., each substrate was used per 8 pots. The pots were placed in a hotbed and seeds of spinach, radish, red beets and carrot were sown into them, and 4-week old lettuce and onion seedlings were planted. The plants were watered with distilled water up to 70 % of capillary water capacity. When the first pair of leaves appeared, eight equal plants were left per pot, except for lettuce planted three heads per pot. The vegetables were harvested at harvest maturity, thoroughly washed under running water and subsequently 25 plants per object were sampled for analysis (lettuce sample was 15 heads). Analyses were conducted on edible parts, which for individual plants were leaves, roots and bulbs.

Dry matter was assessed by drier method at 65 ^{o}C. Lead analysis, after plant material ashing at 450 ^{o}C and dissolving the remains in 10 % nitric(V) acid, was carried out by atomic absorption method [11] in Varian SpectrAA-20 spectrophotometer in acetylene--air flame. Boron was determined by colorimetric curcumin method [12] after plant mineralisation at 550 ^{o}C and ash dissolution in 0.1 mol · dm^{-3} HCl.

Results were statistically elaborated by analysis of variance in a fully randomised design using t-Student test at significance level 0.05. Due to tendencies recurrent in subsequent years and to simplify the result presentation, means for two years were given in the Tables.

Results

Increasing lead content in the substrate caused a raise in lead cumulation by all tested vegetable species (Table 1). Mean Pb content in lettuce leaves cultivated on a control substrate was 10.45 mg · kg^{-1} d.m. It raised by ca 102 % under the influence of 250 mg Pb · kg^{-1} d.m. dose and in result of 500 mg Pb · kg^{-1} d.m. increased by ca 135 % of the initial content.

Table 2

Lead content [mg · kg^{-1} d.m.] in edible parts of vegetables cultivated on substrate with diversified Pb content

Pb addition [mg · kg^{-1} d.m. substrate]	Lettuce		Mean for Pb addition
	Sonata	Marysienka	
0	5.75 a*	15.15 b	10.45 a
250	17.52 c	24.68 e	21.10 b
500	21.05 d	28.00 f	24.52 c
Mean for cultivar variety	14.78 a	22.61 b	
Pb addition [mg · kg^{-1} d.m. substrate]	Spinach		
	Asta F_1	Matador	
0	9.32 a	17.85 b	13.59 a
250	26.02 c	31.82 d	28 92 b
500	33.76 d	42.20 e	37.98 c
Mean for cultivar variety	23.04 a	30 62 b	

Table 2 contd.

Pb addition [mg · kg^{-1} d.m. substrate]	Radish		
	Carmen	Saxa	
0	7.90 a	6.40 a	7.15 a
250	18.52 b	21.62 b	20.08 b
500	31.25 c	40.25 d	35.75 c
Mean for cultivar variety	19.22 a	22.76 a	
Pb addition [mg · kg^{-1} d.m. substrate]	Onion		
	Efekt	Rawska	
0	4.10 a	2.98 a	3.54 a
250	6.30 bc	4.80 ab	5.55 b
500	7.28 cd	8.48 d	7.88 c
Mean for cultivar variety	5.89 a	5.42 a	
Pb addition [mg · kg^{-1} d.m. substrate]	Red beets		
	Glob F_1	Egipski	
0	7.15 a	20.08 b	13.61 a
250	22.42 c	37.38 e	29.90 b
500	30.70 d	43.90 f	37.30 c
Mean for cultivar variety	20.09 a	33.78 b	
Pb addition [mg · kg^{-1} d.m. substrate]	Carrot		
	Kamila F_1	Kalina F_1	
0	4.72 a	5.52 a	5.12 a
250	22.48 b	24.65 b	23.56 b
500	38.05 c	41.32 d	39.69 c
Mean for cultivar variety	21.75 a	23.83 b	

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

Mean content of lead in spinach leaves harvested from the substrate with 0 mg Pb · kg^{-1} d.m. was 13.59 mg Pb · kg^{-1} d.m. and increased by ca 113 % and by ca 180 % after application of respective lead doses. In radish roots from lead-free substrate on an average 7.15 mg · kg^{-1} d.m. of this metal was detected. Supplying subsequent doses of Pb to the substrate resulted in its increased content in radish by ca 181 % and by ca 400 % in comparison with the control material. Onion growing in "clean" substrate revealed on an average 3.54 mg Pb · kg^{-1} d.m. Following the introduction of 250 mg Pb · kg^{-1} d.m. of substrate, this metal concentration in onion increased on average by 57 %, and by 123 % when the dose was increased twice. In red beetroots cultivated on control substrate on average 13.61 mg Pb · kg^{-1} d.m. was found. Addition of 250 and 500 mg Pb · kg^{-1} d.m. of the substrate resulted in its elevated content in roots by about 120 % and 174 % in comparison with beets grown on substrate without lead addition. In carrot roots harvested from the control substrate mean Pb content of 5.12 mg · kg^{-1} d.m.

was found. After supplying subsequent lead doses to the substrate, this element level in carrots raised on average by 360 % and 675 % in comparison with roots from the substrate without Pb addition.

Analysis of boron contents in edible parts of six studied vegetable species in each case demonstrated an elevated content of this element following an increase in lead concentration in the substrate (Table 3).

Table 3

Boron content [mg · kg^{-1} d.m.] in edible parts of vegetables cultivated on substrate with diversified Pb content

Pb addition [mg · kg^{-1} d.m. substrate]	Lettuce		Mean for Pb addition
	Sonata	Marysienka	
0	22.00 a*	31.15 cd	26.58a
250	26.20 b	32.10 d	29.15b
500	28.85 c	34.80 e	31.83c
Mean for cultivar variety	25.68a	32.68b	
Pb addition [mg · kg^{-1} d.m. substrate]	Spinach		
	Asta F_1	Matador	
0	45.80 a	47.35 a	46.58 a
250	47.00 a	47.90 a	47.45 a
500	56. 70 b	49.30 a	53.00 b
Mean for cultivar variety	49.83 a	48.18 a	
Pb addition [mg · kg^{-1} d.m. substrate]	Radish		
	Carmen	Saxa	
0	29.00 b	24.65 a	26.83 a
250	31.90 c	24.50 a	28.20 b
500	38.00 d	28.85 b	33.43 c
Mean for cultivar variety	32.97 b	26.00 a	
Pb addition [mg · kg^{-1} d.m. substrate]	Onion		
	Efekt	Rawska	
0	17.35 a	18.45 a	17.90 a
250	22.25 b	22.10 b	22.18 b
500	26.00 c	23.80 bc	24.90 c
Mean for cultivar variety	21.87 a	21.45 a	
Pb addition [mg · kg^{-1} d.m. substrate]	Red beets		
	Glob F_1	Egipski	
0	20.85 a	21.70 ab	21.28 a
250	24.00 bc	24.90 c	24.45 b
500	30.50 c	29.60 d	30.05 c
Mean for cultivar variety	25.12 a	25.40 a	

Table 3 contd.

Pb addition [mg · kg^{-1} d.m. substrate]	Carrot		
	Kamila F_1	Kalina F_1	
0	22.80 a	26.65 b	24.73 a
250	24.75 ab	28.95 c	26.85 b
500	29.40 c	33.10 c	31.25 c
Mean for cultivar variety	25.65 a	29.57 b	

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

In lettuce leaves from the control soil on average 26.58 mg B · kg^{-1} d.m. was assessed. After supplying 250 and 500 mg Pb · kg^{-1} d.m. to the substrate, boron content in lettuce increased by 9.7 % and 19.8 %, respectively, in comparison with lettuce grown on substrate without lead addition. In spinach leaves grown in control substrate, on average of 46.58 mg B · kg^{-1} d.m. was registered. Supplying 250 mg Pb · kg^{-1} d.m. did not cause any significant changes in spinach boron content and only in the effect of 500 mg Pb · kg^{-1} d.m. added to the substrate, boron content increased by 13.8 % in relation to the material from uncontaminated soil. In radish roots from control substrate on average 26.83 mg B · kg^{-1} d.m. was detected. This element concentration grew with increasing lead doses in the soil by 5.1 % and 24.6 %, respectively. Onion from the control substrate had on average 17.90 mg B · kg^{-1} d.m. This element level raised by an average 23.9 % in effect of 250 mg Pb · kg^{-1} d.m. dose and by 39.1 % under the influence of 500 mg Pb · kg^{-1} d.m. dose. Mean of 21.28 mg B · kg^{-1} d.m. was registered in red beets harvested from the substrate without Pb addition. The dose of 250 mg Pb · kg^{-1} d.m. added to the soil resulted in a raised boron content in beetroots by 14.9 % whereas the double dose caused a 41.2 % increase. In carrot from the control soil on average 24.73 mg B · kg^{-1} d.m. was registered. This element content was increasing with increasing lead content in the substrate by 8.6 % and 26.4 %, respectively.

Discussion

Lead supplied into the soil in doses of 250 and 500 mg · kg^{-1} d.m. produced a manifold increase in this metal cumulation by edible parts of the tested plant species. At the same time growing lead content in tissues was accompanied by increasing boron content, although the response to toxic effect of this metal depended on individual plants. The largest extension of the boron content was observed in onion and red beets, the smallest in case of leave vegetables, lettuce and spinach, was noted. Chosen cultivars of lettuce, spinach, red beets, and carrot differed in intensity of lead accumulation. About the diverse taking this metal by cultivars of the same species was written already earlier [13]. The content of boron in cultivars of three species: lettuce, radish and carrot also differed. However it was not successful to find the proportionality between the quantity of accumulation the lead and boron absorbed by plants.

As a rule lead cumulation causes bigger or smaller decrease in the content of many macro and microelements in plant cells. Some authors under such conditions observed also elevated levels of some elements, particularly potassium, calcium, phosphorus or magnesium. These observations were usually due to some specific features of plant metabolism [4] or defence mechanism [8]. Only few authors have mentioned the effect of lead on boron content in plants. Walker et al [14] determined boron in maize cultivated in pots with an addition of 125 and 250 mg Pb $\cdot$ kg^{-1} d.m., however they did not obtain any consistent results.

The mass of tested plants was not reduced, so growth of boron content in their tissues could not be explained by the simple condensation effect. Another hypothesis is possible. Basic mechanisms of tolerance to heavy metals, including lead, involve their detoxication on the cell wall and preventing their penetration to the protoplast [15]. A subsequent and first living structure on the way of these metals is cell membrane, whose integrity necessary for keeping a cell alive may be ensured only when all damage is quickly repaired [16]. Boron plays an important role in maintaining the proper structure of both cell wall and cell membrane. It is considered a stabiliser of pectin cell wall lattice, where it occurs in stiff polysaccharide complexes as diester with cis-diole sugar groups [17, 18], it also plays a role of regulator of apertures in cell wall [19]. Boron has a decisive share in forming membrane structure [20]. It maintains their efficiency and its deficiency changes cell membrane permeability for sugars, proteins and phosphates [17]. Boron stimulates leaf biosynthesis of glutathion, the important factor of metal detoxification, as well [21].

So, it seems possible that increase in boron uptake under conditions of lead toxicity is caused by its share in cell defensive system. Detailed studies on cell level will undoubtedly further clarify the observations presented in this work.

Conclusions

1. Lead supplied into the soil caused a raise in this metal accumulation by all tested vegetable species (lettuce, spinach, radish, onion, beetroots, carrot), by 57 % to 360 % under influence of 250 mg Pb $\cdot$ kg^{-1} d.m., and by 123 % to 675 % when the dose was increased twice to 500 mg Pb $\cdot$ kg^{-1} d.m.

2. After supplying Pb to the substrate, boron content increased in edible parts of vegetables by 0 % to 23.9 % after application of lower dose, and by 13.8 % to 41.2 % after application of higher one.

3. It seems possible that increase in boron uptake under conditions of lead toxicity is caused by its share in cell defensive system, but this hypothesis requires further detailed investigations.

References

[1] Burzyński M. and Grabowski A.: Acta Soc. Bot. Pol. 1984, **53**(1), 77–86.
[2] John M.K. and Van Laerhoven C.: J. Environ. Qual. 1972, **1**(2), 169–171.
[3] Gawęda M.: Zesz. Nauk. AR w Krakowie, Ser. Rozprawy 1998, **240**, 67–68.
[4] Trivedi S. and Erdei L.: Physiol. Plant. 1992, **84**, 94–100.

[5] Burzyński M.: Acta Physiol. Plant. 1987, **9**(4), 229–238.
[6] Baluk A., Kociałkowski W.Z. and Komisarek J.: Prace Nauk. IOR 1988, **30**(1–2), 173–180.
[7] Godbold D.L. and Kettner C.: J. Plant Physiol. 1991, **139**, 95–99.
[8] Antosiewicz D.M.: Environ. Exp. Bot. 1993, **33**(4), 575–589.
[9] Kannan S. and Keppel H.: Zeitsch. Naturforsch. 1976, **31**, 393–396.
[10] Kabata-Pendias A. and Pendias H.: Biogeochemia pierwiastków śladowych. PWN, Warszawa 1993, pp. 76–80.
[11] Jędrzejczak R. and Szteke B.: Roczn. PZH 1990, **41**, 223–229.
[12] Evans S. and Krahenbuhl U.: Fres. J. Anal. Chem. 1994, **349**, 454–459.
[13] Michalik B., Barański R., Żukowska E. and Gawęda M.: Acta Hort. 1995, **379**, 213–220
[14] Walker W.M., Miller J.E. and Hasset J.J.: Commun. Soil Sci. Plant Anal. 1977, **8**(1), 57–66.
[15] Wierzbicka M.: Acta Soc. Bot. Polon 1995, **64**(1), 81–90
[16] Hall I.L.: J. Exp. Bot. 2002, **53**(336), 1–11.
[17] Power P.P. and Woods W.G.: Plant Soil 1997, **193**, 1–13.
[18] Tanaka Mayuki and Fujiwara Toru.: Environ. J. Physiol. 2008, **456**, 671–677.
[19] Hu H. and Brown P.: Plant Physiol. 1994, **105**, 681–689.
[20] Brown P.H., Bellaloui N., Wimmer M.A., Bassil E.S., Ruiz J., Hu H., Pfeffer H. and Dannel F.: Plant Biol. 2002, **4**, 205–223.
[21] Ruiz J.M., Brebones G., Baghour M., Ragala L., Balakbir A. and Romero L.: Phytochemistry 1998, **48**(2), 269–272.

WPŁYW KUMULACJI OŁOWIU NA ZAWARTOŚĆ BORU W NIEKTÓRYCH WARZYWACH

Katedra Warzywnictwa z Ekonomiką Ogrodnictwa
Uniwersytet Rolniczy w Krakowie

Abstrakt: Sześć gatunków roślin warzywnych: sałatę *Lactuca sativa* L., szpinak *Spinacia oleracea* L., rzodkiewkę *Raphanus sativus* L. subvar. *radicula* Pers., cebulę *Allium cepa* L. var. *cepa*, buraki ćwikłowe *Beta vulgaris* L. i marchew *Daucus carota* L. uprawiano w wazonach na podłożu z dodatkiem ołowiu w ilości: 0, 250 i 500 mg $\cdot$ kg^{-1} s.m.

Zwiększenie zawartości ołowiu w podłożu spowodowało jego intensywną kumulację w jadalnych częściach testowanych gatunków warzyw. Równocześnie wzrosła zawartość boru w tkankach wszystkich analizowanych roślin: w liściach sałaty o 9,7 % pod wpływem dawki 250 mg Pb $\cdot$ kg^{-1} s.m. podłoża i o 19,8 % pod wpływem dawki 500 mg Pb $\cdot$ kg^{-1} s.m., w liściach szpinaku o 13,8 % (tylko pod wpływem większej dawki Pb), w zgrubieniach rzodkiewki o 5,1 i 24,6 %, w cebuli o 23,9 i 39,1 %, w korzeniach buraków o 14,9 i 41,2 %, w korzeniach marchwi o 8,6 i 24,6 % odpowiednio pod wpływem dodatku 250 i 500 mg $\cdot$ kg^{-1} s.m. podłoża.

Słowa kluczowe: kumulacja ołowiu, bor, sałata, szpinak, rzodkiewka, cebula, burak ćwikłowy, marchew