Effect of spring wheat (*Triticum aestivum* L.) treatment with brassinosteroids on the content of cadmium and lead in plant aerial biomass and grain

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ABSTRACT

Spring wheat var. Vánek was cultivated in pots in a soil naturally contaminated with heavy metals. Experimental plants were treated with three different types of brassinosteroids (BRs; 24-epibrassinolide, 24-epicastasterone and 4154) during two different growth stages 29–31 DC (off shooting) and 59–60 DC (beginning of anthesis). Content of heavy metals (Cu, Cd, Pb and Zn) was determined using AAS method in the plant growth stages 47–49 DC (visible awns), 73–75 DC (30–50% of final grain size) and 90–92 DC (full ripeness). At the stages 47–49 DC and 73–75 DC, the content of the heavy metals was determined in the biomass of whole plants, while at the stage 90–92 DC it was determined separately in straw and grains. After the treatment of plants with BRs a decrease in heavy metals content was observed in the growth stage 73–75 DC (i.e. during the period when the plants are harvested for ensilage purposes. Likewise, a decrease of lead content in the grains by 70–74% in the plants treated at both stages 29–31 DC and 59–60 DC and by 48–70% in the plants of the third group (plants treated at stage 59–60 DC) was determined as compared with the untreated plants.

Keywords: spring wheat; brassinosteroids; copper; cadmium; lead; zinc

Brassinosteroids (BRs) are natural plant polyhydroxysteroids supporting plant growth; their structure resembles animal steroid hormones. Brassinosteroids were classified as essential plant hormones nearly 20 years after the discovery of brassinolide (the first brassinosteroid) by Grove et al. (1979) in the rape (*Brassica napus* L.) pollen. Occurrence of brassinosteroids was demonstrated in many plant species including higher and lower plants and at the same time they were detected in parts of plants, e.g. pollen, seeds, leaves, stems, roots and flowers (Sakurai et al. 1999). In 2003, 70 compounds belonging to the class of brassinosteroids were characterized; among them 65 in free form and 5 conjugated (Bajguz and Tretyn 2003).

Brassinosteroids are phytohormones with pleiotropic effects. They influence growth, seed germination, cell elongation, photomorphogenesis and senescence (Upreti and Murti 2004).

In relation to the growth and growth regulators, the typical effect of BRs is coincidental elicitation of cell prolongation and division (Worley and Mitchell 1971). Investigations confirm the ability of brassinosteroids to affect quantitatively plant morphogenesis; this leads to the enhancement of number and growth productive lateral shoots and branches and thereby also to the enhancement of the number of spikes, pods etc. (Sakurai et al. 1999). They also help to overcome stresses provoked by low or high temperature, drought, salt, infection, pesticides and heavy metals (Bajguz 2000, Cao et al. 2005, Janeczko et al. 2005, Kagale et al. 2007, Sharma and Bhardwaj 2007a, b, Ali et al. 2007, 2008).

Heavy metals give rise to antioxidant stress and BRs can effectively reduce it and induce enhancing of antioxidants under heavy metal stress (Hayat et al. 2007). In terms of the affecting of the uptake

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of minerals after treatment with BRs, an increase of the content of minerals in aerial plant biomass was demonstrated (Pirogovskaya et al. 1996, Nafie and El-Khallal 2000, Ageeva et al. 2001) as well as the BRs' ability to decrease uptake of heavy metals (Khripach et al. 1999, Bajguz 2000) and accumulation of radioactive elements (Cs, Sr) by plants (Khripach et al. 1996).

The aim of this work was to evaluate the ability of selected BRs to lessen the uptake and accumulation of four heavy metals (Cd, Pb, Zn and Cu) in spring wheat plants cultivated in a contaminated soil from a polluted area. Content of heavy metals was investigated in biomass, grains and straw of treated and control plants.

MATERIAL AND METHODS

Plant material and conditions of cultivation. Spring wheat, Vánek variety (maintenance of variety: Lochow-Petkus, GmbH, Germany, producer: Selekta, Inc., Czech Republic) was cultivated for two years (2006, 2007) in pots in the outside environment. Plants were cultivated in the soil anthropogenically contaminated with heavy metals from the Příbram location, Central Bohemia, historically polluted from metal ores mining and smelting activities. Average content of minerals in contaminated soil are given in Table 1. Sowing was performed into the pots of 5 l volume filled with 5 kg of homogenized soil. Each pot was fertilized with the same dose of NPK (1.43 g N in the NH_4NO_3 form, 0.16 g P and 0.40 g K in the K_2HPO_4 form). The final number of plants in a pot was twenty. Plants were irrigated with demineralised water.

Weather conditions in cultivation period (from April to July) were similar in both years. Mean air temperature in both years was higher compared with the long-term normal. Mean precipitation in 2006 was higher in April, May and June, lower in July compared with the long-term normal. In April and May 2007 mean precipitation was by 25% lower than normal and in June and July it was higher compared with the long-term normal.

Brassinosteroids and their treatment pattern. Plants were treated with three brassinosteroids (24-epibrassinolide, 24-epicastasterone, and 4154) in two different growth stages (as mentioned below). All BRs were applied in the form of 1 nmol/l of efficient compound in the water solution by spraying of all aerial biomass. Application of BRs was performed in four parallel replicates in each brassinosteroid. Applied brassinosteroids (Figure 1) were synthesized in the Institute of Organic Chemistry and Biochemistry of the Academy of Sciences of the Czech Republic. 24-epibrassinolide (24-epiBL) and 24-epicastasterone (24-epiCS) are naturally occurring phytohormones and compound 4154 is a synthetic brassinosteroid registered in the Czech Republic (No. 294343, conferred on 4/10/2004) and the EU (No. 1401278, conferred on 28/9/2005). Plants or experimental pots were divided before the application of BRs into four groups that differed in growth stage, in the date of treatment and number of BRs applications (Table 2).

Sampling of plant material. Sampling from the experimental pots was performed three weeks after the application of brassinosteroids in plant growth stages referred to a decimal code for the growth stages of cereals. The first sampling was performed in the plant growth stage 47–49 DC (visible awns), the second sampling in growth stage 73–75 DC (30–50% of final grain size). Grain and straw samples were taken in the growth stage 90–92 DC (full ripeness). Green plants were taken from the experimental pots, cleaned up with distilled water and subsequently freeze-dried.

Chemical and laboratory materials and equipment. Nitric acid (HNO_3, 65%, p.p., Lachema Neratovice CZ and Suprapur® Merck, Germany) and demineralised water (corresponding to quality degree 1 according to EN ISO 3696 for the calibration of ICP-OES) were used for the dry decomposition of plant samples. For the calibration of AAS and testing of the method of modified dry decomposition the following materials were used: calibration solutions with one element (Analytika, Ltd., CZ) 1.000 ± 0.002 g/l in 2% HNO_3 for elements Cu, Pb and Zn, while cadmium was dissolved in 2% HCl; certified reference material

Table 1. Content of selected metals and characteristics of used soil contaminated with heavy metals from the district of Příbram, Czech Republic

CEC (mmol/kg)	pH_{KCl}	C_{ox} (%)	Zn	Cu	Cd	Pb
			mg/kg DM			
123	4.52 ± 0.02	1.91 ± 0.006	187 ± 8.0	42.7 ± 2.0	3.60 ± 0.17	1321 ± 71

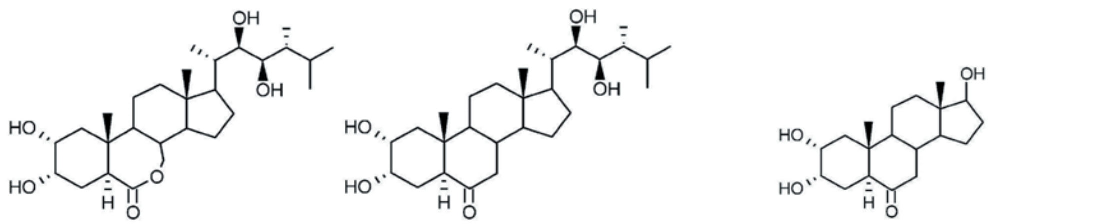

Figure 1. Chemical structure of brassinosteroids used for wheat treatment

NIST 8436 (Durum Wheat Flour) and internal reference material (IRM) from International Plant Analytical Exchange (IPE), RM Sample 3, Wheat 684, Quarterly Report 2000.3.

Dry decomposition of samples was performed with muffle furnace LM 112.10, heating plate ALTEC® JRT 350 with temperature graduation per 10°C, and ultrasonic bath Elma Transonic T660/H.

Analyses of metals were performed by atomic spectrometer VARIAN SpectrAA 110 with the possibility of emission spectra measuring and Varian SpectrAA 280Z atomic absorption spectrometer furnished with GTA 120 electrothermic atomizer.

Table 2. Variants of analyzed spring wheat plants treated with BRs at different growth stages

Treatment*	Stage of BRs application DC	
	29–31	59–60
D (control)	–	–
A-I	+	–
B-I	+	–
C-I	+	–
A-II	+	+
B-II	+	+
C-II	+	+
A-III	–	+
B-III	–	+
C-III	–	+

*1st group of plants (pots A-I, B-I, C-I) was treated with brassinosteroids A (24-epibrassinolide), B (24-epicastasterone) and C (4154) once at the growth plant stage according to Zadoks growth scale 29-31 DC (off shooting); 2nd group (pots A-II, B-II, C-II) was treated with brassinosteroids two times, firstly in the plant growth stage 29-31 DC and again in the plant growth stage 59-60 DC (beginning of flowering); 3rd group (pots A-III, B-III, C-III) was treated once in the plant growth stage 59-60 DC (beginning of flowering); 4th group (D) consisted of untreated control plants

Dry decomposition procedure. Samples of grain, straw and freeze-dried green plants were mineralized before analyses by dry thermal decomposition using SOP-3C (Standard Operation Procedure for Dry Decomposition of Higher Plants and Green Algae) (Mader et al. 1998). Before dry decomposition, samples of freeze-dried plants, straw and grains were roughly ground in an IKA A11 Basic mill equipped with stainless steel working parts. Weight of the homogenized sample was about 1 g and each sample was analysed in two replicates. Initial temperature of the heater plate was 150°C, final temperature was 350°C. After cooling, the samples were combusted in a muffle oven at 480°C; the ash was dissolved in 1.5 ml conc. HNO_3 (65% p.a.) and then repeatedly combusted at 480°C. After the combustion, the samples (white ash) were dissolved in 5 ml of 1.5% HNO_3 after the addition of 1 ml conc. HNO_3 (65% p.a.).

FAAS determination (flame atomic absorption spectrometry) and ET-AAS (atomic absorption spectrometry with electrothermic atomization). Determination of Cd, Zn and Cu was performed with flame atomic absorption spectrometry in samples prepared with dry decomposition. Atomization of samples was proceeded in the flame acetylene/air; rate of injection of samples into the flame was 4.5 ml/min. Wavelengths used for the metals determination were 228.8, 324.8 and 213.9 nm for Cd, Cu and Zn, respectively. Determination of Pb was performed with atomic absorption spectrometry with electrothermic atomization by Varian SpectrAA 280Z atomic absorption spectrometer furnished with GTA 120 electrothermic atomizer at wavelength 283.3 nm.

Determination of dry weight. Dry matter of straw samples was determined by drying at 105°C in a laboratory oven and that of grain samples at 130°C to constant weight (ISO 712).

Replicates and statistic analysis. All variants were cultivated and treated in four replicates. Statistical evaluation was performed with ANOVA (LSD-test, $P < 0.05$).

RESULTS AND DISCUSSION

Experimental plants of the first group (A-I, B-I and C-I) and the second one (A-II, B-II and C-II) treated with BRs in the plant growth stage 29–31 DC did not differ in the growth stage 47–49 DC from untreated control and the plants of the third group (not treated in the stage 29–31 DC). The first differences in the content of investigated metals were shown in the plant growth stage 73–75 DC (Table 3).

A distinct trend in copper content was not observed in the plant biomass. Content of lead decreased in all variants of treated plants. A decreased lead content was determined in the plants of the second group (as a whole treated two times) and the third (A-III, B-III and C-III) group (as a whole treated ones), in which the last BRs application was performed in growth stage 59–60 DC. In the first group that was treated only once in the stage 29–31 DC, lead content was higher than those in the other two groups. Similarly to lead content in the plants of the second group and the third group, lower cadmium and zinc contents were determined as related to the contents of the first group and in control plants (with the exception of plants treated with 4154 in the third group, where the lower Zn content was not determined).

After the harvest of plants in growth stage 90–92 DC (Table 4), a lower copper content in the first group and the third group (with the exception of plants treated with 4154 in the third group) was determined in plant straw. Likewise in the growth stage 73–75 DC, lower zinc content was determined in all plants of the second group and in the plants of the third group treated with 24-epiBL and 24-epiCS. No significant difference of the cadmium content in the growth stage 90–92 DC was found, with the exception of plants treated with 4154 in the first group and 24-epiBL in the second group. Lower lead content was determined in the plants of the second group and the third group treated with 24-epiBL and 24-epiCS.

Copper content was affected more likely according to the actual and individual status of plants, however, in some cases these physiological processes could be affected by BRs treatment (Figure 2). Zinc content in aerial biomass decreased during plant growth (Figure 3). A significant decrease of cadmium content was determined after the BRs application in the growth stage 73–75 DC in the plants of the second group and the third group (Figure 4). Lead was accumulated in the plant biomass of the control group during the all vegetation period (Figure 5). Lower lead content in the stage 90–92 DC was found only in the plants of the second group and the third group that were treated with 24-epiBL and 24-epiCS. No significant difference was found in the plants of the first group that were treated in the growth stage 29–31 DC and in the treatment with brassinosteroid 4154.

After the application of BRs, lead content in grains decreased in the second and the third group (Table 5). While copper content significantly decreased in the plants of the third group following 24-epiCS treatment, the decrease of copper content was not statistically significant in other variants. Effect of brassinosteroids on the content of metals in grains of control plants is shown in Figure 6.

Table 3. Content of Cu, Zn, Cd and Pb in plants treated with brassinosteroids and in untreated control at growth stage 73-75 DC (mg/kg DM)

	Copper	Zinc	Cadmium	Lead
D (control)	2.71	123	6.48	5.28
A-I	3.59*	130	5.91	3.59*
B-I	3.35	129	5.42	2.37*
C-I	1.60*	128	5.06*	3.39*
A-II	3.02	96.8*	4.01*	2.33*
B-II	3.71*	100*	5.04*	1.70*
C-II	1.63*	108*	4.10*	2.95*
A-III	2.52	92.0*	3.14*	1.36*
B-III	1.94	93.5*	3.01*	1.88*
C-III	3.56*	112	5.08*	2.68*

Table 4. Content of Cu, Zn, Cd and Pb in plants treated with brassinosteroids and in untreated control at growth stage 90-92 DC (mg/kg DM)

	Copper	Zinc	Cadmium	Lead
D (control)	3.07	55.2	26.1	5.30
A-I	2.17*	47.4	29.3	4.70
B-I	2.30*	46.8	30.6	4.80
C-I	2.28*	46.0	31.7*	4.54
A-II	2.48	33.7*	19.9*	3.56*
B-II	2.98	41.8*	26.5	3.65*
C-II	2.64	34.2*	25.6	4.45
A-III	2.08*	26.3*	22.4	3.48*
B-III	1.75*	26.2*	22.7	2.57*
C-III	2.36	47.0	26.6	4.54

*Statistically significant difference related to untreated control; for the used symbols of experimental variants see Table 2

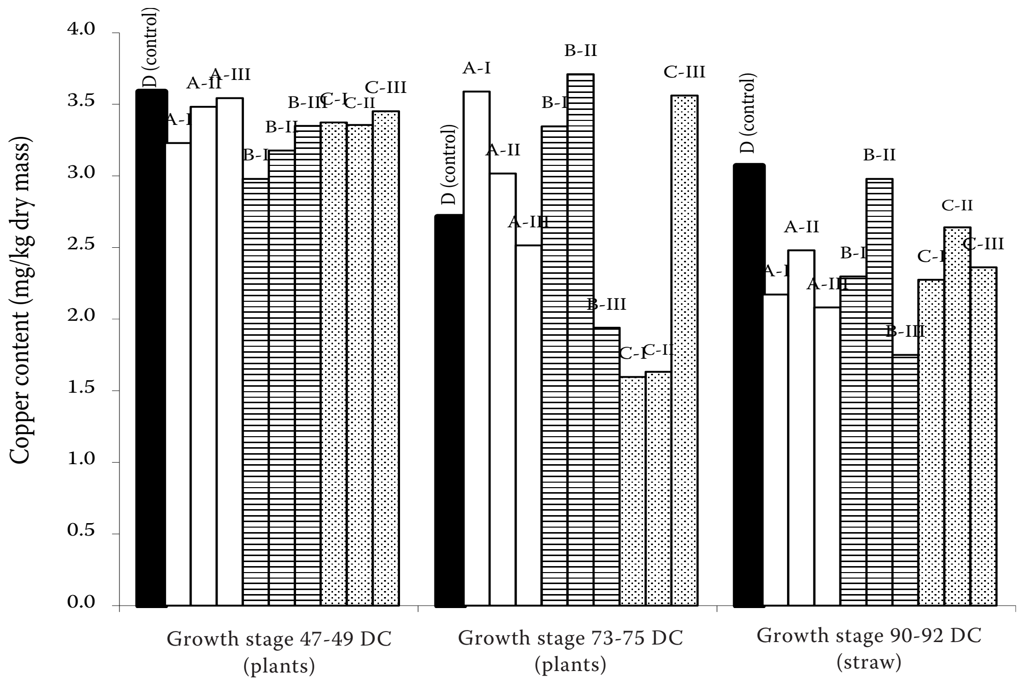

Figure 2. Content of Cu in aerial biomass of plants (mg/kg DM)

Enhanced resistance of BR-treated plants to extreme temperature, salt, pathogens and environmental stresses (heavy metals) was reported by Krishna (2003). The present study revealed the effect of BRs treatment on the accumulation of Cd, Cu, Pb and Zn contents in aerial wheat biomass or grains. The obtained results are in agreement with the results of Bajguz (2000) who observed that 24-epiBL at the concentration of 10^{-8} mol/l in combination with heavy metals blocked metal accumulation in algal cells. At metal concentrations of 10^{-6}–10^{-4} mol/l, a combination with 24-epiBL appeared to have a stronger stimulatory effect on a number of cells than a single metal (a stronger inhibitory effect). The inhibitory effect on metal accumulation of 24-epiBL mixed with different heavy metals was arranged in the following order: zinc > cadmium > lead > copper. Our results obtained for spring wheat as an important crop confirm and are complementary to the results of Sharma and Bhardwaj (2007a, b), which describe the effects of 24-epiBL on plant growth, heavy

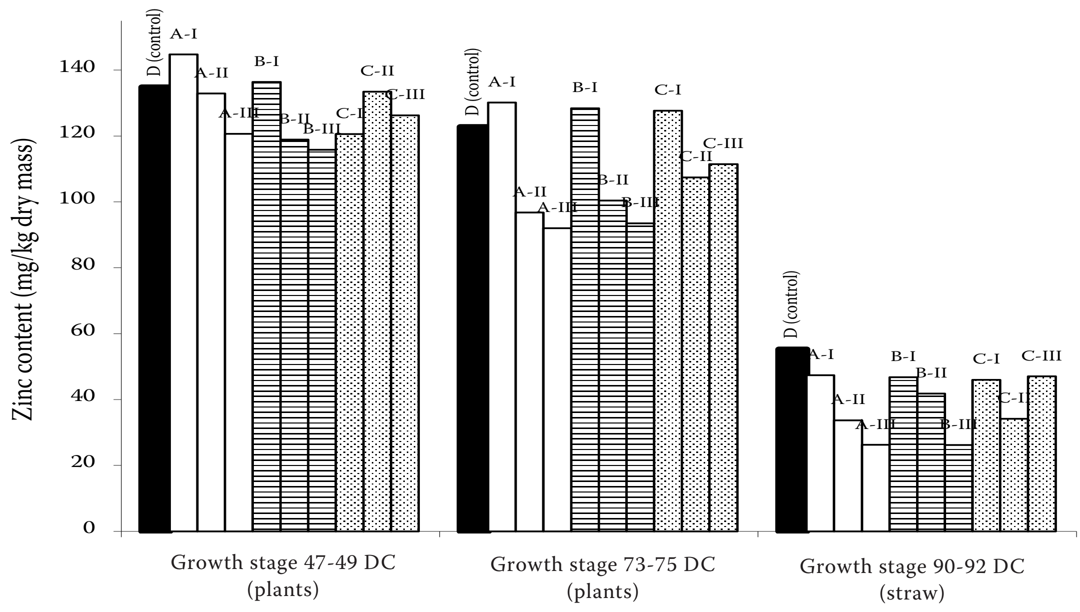

Figure 3. Content of Zn in aerial biomass of plants (mg/kg DM)

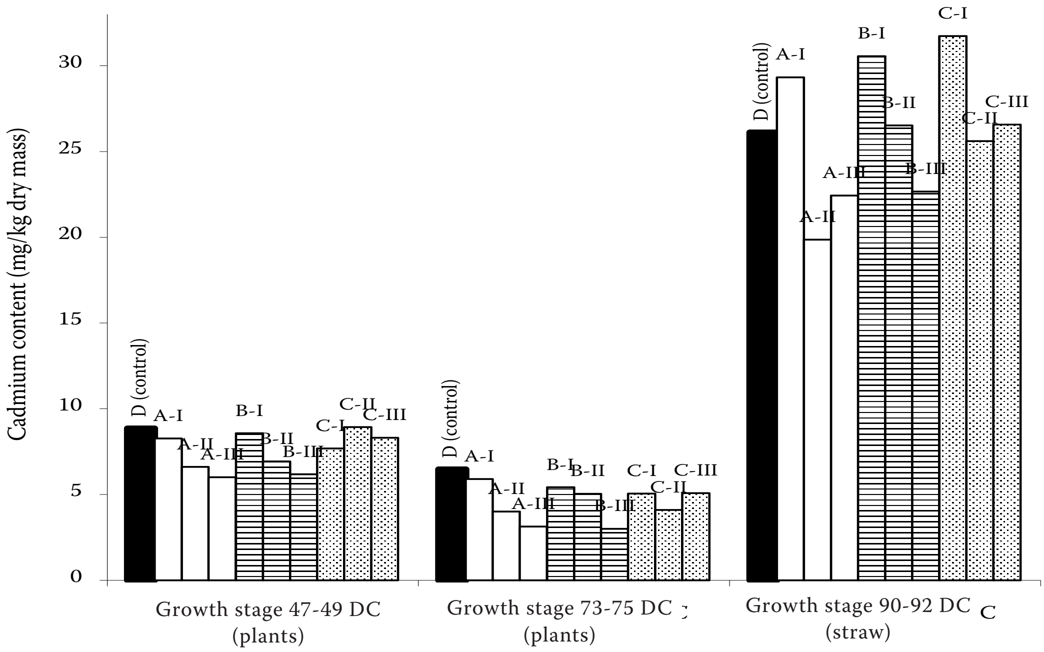

Figure 4. Content of Cd in aerial biomass of plants (mg/kg DM)

metals uptake in the plants of *Brassica juncea* L. under heavy metal (Zn, Cu, Mn, Co and Ni) stress. 24-epiBL after the pre-germination treatment blocked copper metal uptake and accumulation in the plants. Likewise results of Anuradha and Rao (2007), obtained in a study on radish (*Raphanus sativus* L.) after the treatment with 24-epiBL and 28-homobrassinolide, and clearly indicated the inhibitory influence of brassinosteroids on the cadmium toxicity. BRs supplementation alleviated the toxic effect of cadmium and increased the percentage of seed germination and seedling growth. The application of BRs at low concentrations at a certain stage of development reduced significantly the metal absorption in barley, tomatoes and sugar beet (Volynets et al. 1997). Our results indicate that for the decrease of heavy metals content in plants after the BRs application the growth stage of spring wheat is very important.

The present study shows that the content of heavy metals in wheat plants is reduced variously in different growth stages. The plants of the second group and the third group contained in biomass at the growth stage 73–75 DC lower Pb content

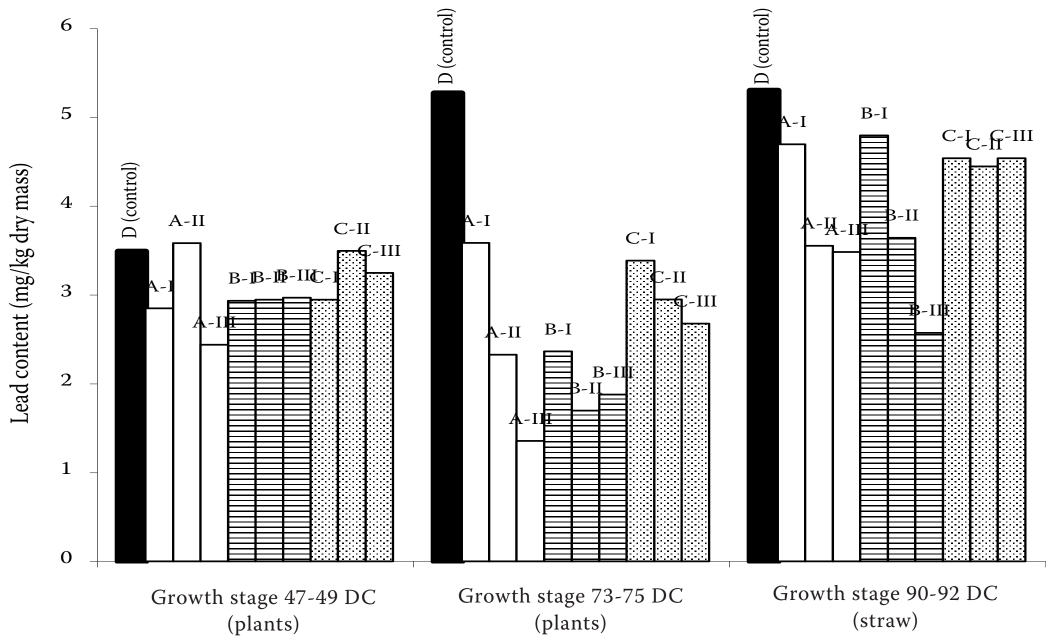

Figure 5. Content of Pb in aerial biomass of plants (mg/kg DM)

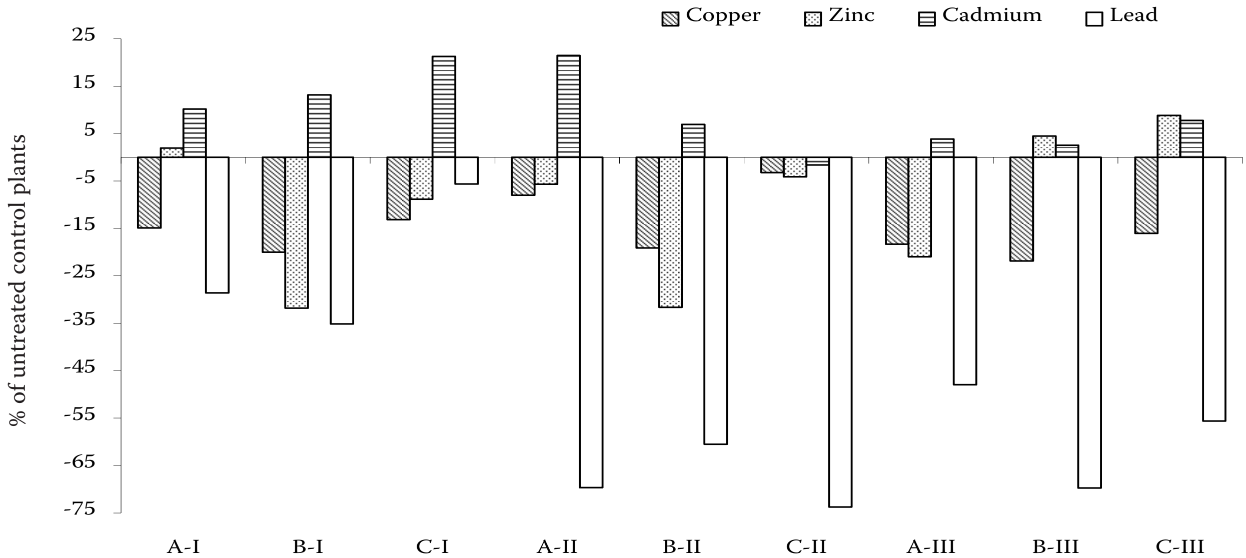

Figure 6. Content of Cu, Zn, Cd and Pb in the grains of treated plants (% of untreated plants)

as compared to control plants and the plants of the first group, which was treated with BRs last at the growth stage 29–31 DC. Also in the plants of the second group and the third group at the growth stage 73–75 DC lower Cd and Zn contents were determined (with the exception of brassinosteroid 4154 in the third group). The treatment of wheat plants with brassinosteroids 24-epiBL, 24-epiCS and 4154 at the plant growth stage 29–31 DC did not significantly influence content of the heavy metals in aerial plant biomass at the growth stage 47–49 DC. In the straw at the growth stage 90–92 DC, lower Pb and Zn contents were subsequently determined only in the plants treated with 24-epiBL and 24-epiCS (Zn also with the application of 4154 in the second group). Lower Cd content was determined only in the variant treated two times with 24-epiBL, which was considered as a highly active brassinosteroid. Lower Pb content was found in the grains of plants of the second group (treated two times in the stages 29–31 DC and 59–60 DC) and the third group (treated once in the stage 59–60 DC); however, no decrease of Zn and Cd contents in grain was found. In terms of the content of heavy metals related to the number and growth stage of BRs applications, the most effective variants of treatment leading to decrease of metal content proved either double treatments in the growth stages 29–31 DC and 59–60 DC (plants of the second group) or one treatment only in the stage 59–60 DC (plants of the third group).

Table 5. Content of Cu, Zn, Cd and Pb in grain of plants treated with brassinosteroids and in untreated control (mg/kg DM)

	Copper	Zinc	Cadmium	Lead
D (control)	4.88	15.6	11.7	1.87
A-I	4.15	15.9	12.9	1.33
B-I	3.90*	10.6	13.2	1.21
C-I	4.24	14.2	14.2*	1.76
A-II	4.49	14.7	14.2*	0.57*
B-II	3.95*	10.7	12.5	0.74*
C-II	4.72	14.9	11.5	0.49*
A-III	3.98*	12.3	12.1	0.97*
B-III	3.81*	16.3	12.0	0.57*
C-III	4.10*	17.0	12.6	0.83*

*statistically significant difference related to untreated control; for the used symbols of experimental variants see Table 2

In conclusion, from the point of view of final effect on the content of the heavy metals in plant biomass and grains, the most suitable variant appears to be the single treatment at the growth stage 59–60 DC, which is economically preferable and its final effect does not differ remarkably from double treatments. After BRs treatment of plants, a decrease of heavy metals in the plant biomass in the growth stage 73–75 DC (i.e. on the date when the plants are usually harvested for ensilage) was determined. Likewise lead content in grains decreased in the plants of the second group by 70–74% and of the third group by 48–70%. Thus, treatment of plants with BRs effectively decreased content of cadmium and lead in wheat plants and content of lead in harvested grain and diminished in such way the input of these contaminants into the food chain.

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