Sensitivity of selected crops to lead, cadmium and arsenic in early stages of ontogenesis

Citlivosť vybraných poľnohospodárskych plodín na olovo, kadmium a arzén v skorých štádiách individuálneho vývinu

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Abstract

This paper examines the influence of Pb^{+2}, Cd^{+2} and As^{+3} on growth of roots in legumes (broad bean, soybean, pea) and cereals (barley, maize). Roots of germinating plants were exposed to two different levels of Pb^{+2} (300 and 500 mg*L^{-1}), Cd^{+2} (100 and 300 mg*L^{-1}) and As^{+3} (50 and 100 mg*L^{-1}) during four day experiment. During this time, length of roots were daily measured. Toxicity of metal treatment on plant roots was calculated as phytotoxicity index (IP). In all cases, a moderate effect of lead treatment was observed (IP up to 56.67 %) while higher doses of cadmium and arsenic resulted in increase of IP above 50 %. In cases of barley and maize, the toxic effect of almost all test doses of the heavy metals was observed as soon as 24 hours after their application. Generally, a higher tolerance to tested metals showed roots of both bean cultivars (IP 16.27- 69.53 %), while the most sensitive reactions had roots of barley and soybean (IP > 50 %, excluding dose Pb 300).

Keywords: arsenic, cadmium, cereals, lead, legumes, root growth

Abstrakt

Príspevok sa zameriava na testovanie vplyvu iónov Pb^{+2}, Cd^{+2} a As^{+3} na rast koreňov strukovín (bôb, sója, hrach) a obilnín (jačmeň, kukurica). Korene klíčiacich rastlín boli vystavené dvom rôznym dávkam iónov Pb^{+2} (300 and 500 mg*L^{-1}), Cd^{+2} (100 and 300 mg*L^{-1}) a As^{+3} (50 and 100 mg*L^{-1}) počas štyroch dní experimentu, pričom každých 24 hodín bola meraná dĺžka koreňov. Toxicita aplikovaných dávok kovov na korene rastlín bola stanovená indexom fytotoxicity (IP). V prípade všetkých variantov experimentu bol zaznamenaný miernejší účinok olova (IP do 56.67 %). Vplyvom vyšších dávok kadmia a arzénu došlo ku zvýšeniu IP nad 50 %. V prípade jačmeňa a kukurice bol toxický účinok takmer všetkých testovaných dávok kovov pozorovaný už 24 hodín po ich aplikácii. Všeobecne vyššiu toleranciu voči testovaným kovom vykazovali korene odrôd bôbu (IP 16.27- 69.53 %) a najcitlivešie reagovali korene jačmeňa a sóje (IP > 50 okrem dávky Pb 300).

Kľúčové slová: arzén, kadmium, obilniny, olovo, rast koreňov, strukoviny

Detailný abstrakt

Ťažké kovy a metaloidy patria medzi významné kontaminanty životného prostredia. Zvýšená kumulácia týchto prvkov v pôde spôsobuje zníženie výnosov poľnohospodárskych plodín a ohrozuje jednotlivé články potravinového reťazca. Riešenie danej problematiky spočíva jednak v aplikácii vhodných remediačných technológií a jednak v hľadaní a šľachtení tolerantných odrôd rastlín. Štúdium mechanizmov tolerancie rastlín na rôzne kontaminanty sa často opiera o sledovanie základných parametrov rastu (dĺžka a hmotnosť orgánov). Meraním týchto parametrov možno pomerne rýchlo stanoviť toleranciu veľkého počtu rastlinných druhov voči viacerým druhom kontaminantov.

Príspevok sa zameriava na testovanie vplyvu iónov Pb^{+2}, Cd^{+2} and As^{+3} na rast koreňov strukovín (bôb, sója, hrach) a obilnín (jačmeň, kukurica). Korene klíčiacich rastlín s dĺžkou 6-8 mm boli vystavené dvom rôznym dávkam iónov Pb^{+2} (300 and 500 $mg*L^{-1}$), Cd^{+2} (100 and 300 $mg*L^{-1}$) a As^{+3} (50 and 100 $mg*L^{-1}$) počas štyroch dní experimentu, pričom každých 24 hodín bola meraná dĺžka koreňov. Kontrolnú vzorku predstavovali korene rastlín zaliate destilovanou vodou. Toxicita aplikovaných dávok kovov na korene rastlín bola stanovená indexom fytotoxicity (IP) na základe dĺžky koreňov kontrolných rastlín a rastlín vystavených testovaným dávkam iónov kovov, pričom IP=100 % predstavuje absolútnu toxicitu. Dĺžka koreňov hrachu a sóje bola stanovená iba v štvrtý deň experimentu, nakoľko morfológia koreňov v predchádzajúce dni neumožňovala merania.

Toxický účinok všetkých testovaných dávok kovov bol pozorovaný už 24 hodín po ich aplikácii v prípade jačmeňa a kukurice. Vysoko citlivé boli korene jačmeňa najmä na testované dávky arzénu (IP 64.43 % a 73.47 %). V prípade odrôd bôbu sme zaznamenali miernu toxicitu (IP do 21 %), pričom niektoré dávky kovov pôsobili na korene bôbu dokonca stimulačne. Toxický účinok aplikovaných iónov kovov sa v priebehu ďalších dní zvyšoval, pričom všeobecne miernejší účinok pôsobili dávky olova (IP do 56.67 %). Vplyvom vyšších dávok kadmia a arzénu došlo štvrtý deň experimentu ku zvýšeniu IP nad 50 %. Všeobecne vyššiu toleranciu voči testovaným kovom vykazovali korene odrôd bôbu (IP 16.27-69.53 %) a najcitlivešie reagovali korene jačmeňa a sóje (IP > 50 okrem dávky Pb 300).

Z vizuálnych symptómov toxicity aplikovaných iónov kovov sme okrem skrátenia koreňov zaznamenali hnednutie až černanie koreňov, ktoré sa prejavilo najmä v prípade koreňov bôbovitých rastlín.

Kľúčové slová: arzén, kadmium, obilniny, olovo, rast koreňov, strukoviny

Introduction

Heavy metals (HM) and metalloids (e.g. arsenic which is, for simplicity, ranked among heavy metals further in the text) belong to the most important sorts of contaminant in the environment (Järup, 2003). Agricultural soils in many parts of the world are slightly to moderately contaminated with heavy metals such as Cd, Cu, Zn, Ni, Co, Cr, Pb and As (Yadav, 2010). Heavy metals enter plants especially from the soil solution. It traverses the root through symplastic or apoplastic pathways before entering the xylem and being translocated to the shoot. Growth ihibition is a general phenomenon associated with most of heavy metals (Hall, 2002), while the tolerance limits for HM toxicity are specific for each species and even for each variety of cultural plants (Vasilev and Yordanov, 1997).

Lead (Pb) and cadmium (Cd) are the most abundant metals polluting the environment (Seregin et al., 2004). Both cadmium and lead primarily accumulate in root cells (Benavides et al., 2005; Sharma and Dubey, 2005); according to Wu (1990), about 70–85 % of Cd absorbed by various plants remains in the roots. The toxic effects of these metals is related to its ability to generate reactive oxygen species (ROS) resulting in unbalanced cellular redox homeostasis (Clemens, 2001; Schützendübel et al., 2001). The most common effect of Cd and Pb in plants is inhibition of growth, activation or inhibition of enzymes, reduction of transpiration rate and water content (Benavides et al., 2005; Påhlsson, 1989; Sharma and Dubey, 2005).

Arsenic (As) is a crystalline metalloid that exists in several forms and oxidation states. Its toxicity and mobility in the environment depend on both its chemical form and species (Pongratz,1998). There is no evidence that arsenic (As) is essential for plant growth, although small amounts of arsenic can stimulate plant growth and increase plant biomass (Onken and Hossner, 1995). In addition, small yield increases have been observed at low levels of As, especially for tolerant plants such as corn, potatoes, rye, and wheat (Carbonell et al., 1998; Gulz et al., 2005; Jacobs et al., 1970). However, with increasing concentration As becomes eventually very toxic for all plants, causing chlorosis, necrosis, inhibition of growth and finally death (Zhao et al., 2009). Disturbance of plant mineral nutrition is the main cause for yield decrease, the most frequent sign of As toxicity (Päivöke and Simola, 2001). This is often accompanied by root discoloration and necrosis of leaf tips and margins, indicating inhibition of root water uptake and ultimately resulting in death from wilting (Finnegan and Chen, 2012).

On one hand, heavy metals show negative effects on plants. On the other hand, plants have developed a variety of tolerance mechanisms in response to metal exposure (Cheng, 2003; Hall, 2002; Viehweger, 2014). Metal sequestration in distinct cellular compartments plays a pivotal role in metal tolerance. For this purpose cells provide a coordinated set of transport systems in each cellular membrane (Viehweger, 2014). Another way for enhanced metal tolerance is synthesis and deposition of polysaccharides like, callose or lignin creating a barrier that stops entering through the uptake of large amounts of metals and its sequestration in the vacuole, accompanied by changes in root growth and branching pattern or by its translocation to the aboveground parts of plant (Fahr et al., 2013; Lux et al., 2011).

Examination of plant´s sensitivity to heavy metal ions is carried out by various methods. Measurement of particular organ´s length is relatively simple and fast approach to analyze plants exposed to different conditions of the environment (including heavy metal exposure). This approach is predominantly used in case of higher number of tested plants when it is difficult to monitor and measure indicators such as fresh root´s weight and dry mass. The objective of this study was to study effects of particular heavy metals: lead, cadmium and arsenic on root growth in five plant species, i.e. broad bean, soybean, pea, barley and maize in early stage of ontogenesis.

Materials and Methods

The analysis were performed in the Laboratory of plant stress at Department of Botany and Genetics, Constantine the Philosopher University in Nitra. One cultivar of soybean (*Glycine max* cv. Korada), pea (*Pisum sativum* cv. Olivín), barley (*Hordeum vulgare* cv. Garant), maize (*Zea mays* cv. Quintal) and two broad bean cultivars (*Vicia faba* cv. Aštar and Pieštanský) were used for the analysis.

Seed preparation

Seeds were sterilized for 5 minutes in 75 % ethanol, thereafter 10 minutes in 1 % solution of natrium hypochlorite (NaClO) and rinsed thoroughly with distilled water. Moreover, broad bean and maize seeds had to be immersed in distilled water for 12 hours (at laboratory temperature) for reason of swelling. After washing treatment, seeds were placed on sterilized Petri dishes of 15 cm in diameter, each with a filtrate paper moistened with distilled water used as a culture media. Petri dishes with seeds were transferred to darkness at 25 °C. Sprouted seedlings with 6-8 mm long roots were selected and transferred on new dishes as described in detail elsewhere (Rucinska et al., 2004).

Exposure to heavy metal

Seedlings with 6-8 mm long roots were transferred on new Petri dishes contained a filtrate paper moistened with a heavy metal solution. Seedlings were exposed to two different concentrations of heavy metals: Pb^{+2} (300 and 500 mg*L^{-1}), Cd^{+2} (100 and 300 mg*L^{-1}), As^{+3} (50 and 100 mg*L^{-1}). Heavy metals were applied as compound solutions: $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_2O$ and As_2O_3. In control sample, distilled water was used instead of heavy metals.

Measurement of root length

Root length was measured in plants exposed to stress condition and also non stressed plants (control treatment) every 24 hours during the four-day experiment. 15-20 seeds per treatment were used, and the measurement was performed in three independent experiments (altogether 45-60 seedlings for each treatment). Root length was measured using millimeter paper. In the case of pea and soybean cultivars, root´s morphology did not allow measurement of length of roots from the first to the third day of experiment. On the fourth day of the experiment, the roots straightened to an extent that allowed measurements of their lengths.

Statistical analysis

Experimental data were processed statistically by the Student´s t-test (calculated in MS Excel) at the 0.05 significance level.

Determination of phytotoxicity index

Phytotoxicity index (IP) was calculated according to the following formula (Chou and Lin, 1976):

$$IP\ (\%) = \frac{\text{root length of control} - \text{root length of treatment}}{\text{root length of control}} \times 100$$

Results and discussion

The present study investigate the effect of selected heavy metals on growth of roots in legumes and cereals in early stage of ontogenesis. The reduction of roots´ length was observed as the principal symptom of phytotoxicity (Table 1-3). Blackening appeared especially on faba bean roots treated with higher concentration of cadmium and arsenic which can indicate metal-induced oxidation of different phenols in roots (Fecht-Christoffers et al., 2003).

In our work, lead applied in the form of lead(II) nitrate $Pb(NO_3)_2$ had two types of effects on growth of roots. In some cases (bean cultivars), the application of lower doses of Pb^{+2} had enhanced effect on root growth during the period from the first to the third day of experiment (Table 2). The observed stimulatory effect, appearing after application of the metal solution, is probably a result of the effect of nitrate ions from the applied solution or the dose of the metal itself (Bashmakov et al., 2005; Yogeetha et al., 2004). On the contrary, the application of Pb^{+2} at higher concentration (500 mg*L^{-1}) had a toxic effect on root elongation and overall development of root system (Table 1-3). High sensitivity to tested doses of Pb^{+2} was predominantly observed on barley (IP 23.79 % and 38.04 % respectively), maize (IP 24.36 at higher doses of lead) and bean cv. Piešťanský (IP 20.38 at higher doses of lead) after first 24 hours of experiment. Higher sensitivity to dose of Pb 500 was shown also in soybean roots (IP 53.44, determined on the fourth day of the experiment). In general, low or moderate toxicity of Pb to various plant species including faba bean, maize and pea was reported also by other authors (Ivanov et al., 2003; Påhlsson, 1989; Piechalak et al., 2002; Tung and Temple, 1996).

Cadmium was applied at two concentrations: 100 and 300 mg*L^{-1}. In general, Cd had a harmful influence on root system of tested plants (Table 1-3). The most Cd-affected crop was barley (IP 80.26 % and IP 96.91 % at lower and higher dose of cadmium). High sensitivity to higher doses of cadmium showed also roots of maize (IP 80.2 %). On the contrary, the highest tolerance to test doses of cadmium was shown in roots of faba bean cv. Piesťanský (IP 37.18 % and IP 58.5 % respectively). Relatively high tolerance was observed also at roots of pea at lower concentration of cadmium (IP 44.16 %) (Table 3). Out of tested legumes, soybean showed the lowest tolerance (IP 65.00 % and 65.70 % respectively) (Table 3). Peralta et al. (2001) reported a reduction of shoot length of *Medicago sativa* at the applied dose of 5 mg and 20 mg*L^{-1} Cd^{2+}, while the dose of 40 mg*L^{-1} was lethal. Our results are somewhat different from those by Kuboi et al. (1986), who reported that dicotyledons are more sensitive to Cd than barley and other cereals, which are considered to be semi-resistant. Results of different investigations (including toxicity/tolerance monitoring) are, however, difficult to compare as the nature of heavy metal effect varies not only between the species, but also between genotypes of the same species (Ahmad et al., 2012; Metwally et al., 2005), age of the plants, the concentration and duration of the effect, physical and chemical properties of contaminants (Vassilev and Yordanov,1997) as well as physical and chemical properties of soil (McCully, 1999).

Genotypic differences in tolerance to metal ions were confirmed also through our experiments. Application of Cd^{+2} and As^{+3} at concentration of 100 mg*L^{-1} resulted in different length of roots in both tested cultivars of broad bean. Cultivar Pieštanský had longer roots compared to cultivar Aštar (Table 2).

Although arsenic was applied at the lowest concentration (50 and 100 mg*L^{-1}) compared to other metals (Pb^{+2} 300 and 500 mg*L^{-1}; Cd^{+2} 100 and 300 mg*L^{-1}), this metal had the most toxic effect on development of root system. Lower concentrations of As were used due to predicted toxicity of this element (Piršelová et al., 2009). From tested set of plants, the most negatively influenced crop was barley, because its root system was significantly underdeveloped and growth was severely inhibited on second day after application of heavy metal (Table 1). This fact was significantly evident on the fourth day when the root´s length in non-contaminated barley sample was 86.68 mm, whereas root´s length of As-contaminated seedling (at concentration of 100 mg*L^{-1}) was only 6.06 mm (IP 93.01 %). Very similar result was detected in soybean (IP 92.33 %). Out of the set of tested plants, the crop third most sensitive to arsenic was maize (IP 74.09 and 79.15) (Table 1). According to Evans et al. (2005), arsenic in low doses (100 µg*L^{-1}) can be even beneficial to growth and development of maize. Physiological and biochemical causes for this phenomenon have not been completely clarified yet. Two possibilities exist for growth stimulation by As: first, stimulation of plant systems by small amount of As, second, displacement of phosphate ions from the soil by arsenate ions, with the resultant increase of phosphate availability (Jacobs and Keeney, 1970). A higher tolerance to arsenic was observed in roots of pea and both varieties of bean (IP up to 70.0 %). Despite this fact, the high values of IP point to high sensitivity of these crops to arsenic, which is consistent with results of testing the effect of arsenic on growth of other legumes (Carbonell-Barrachina et al., 1997; Stoeva et al., 2005).

In general, a greater reduction in growth of roots was observed when higher doses of heavy metals was applied. It is in accordance with findings of other authors (Moosavi et al., 2012; Munzuroglu and Zengin, 2006).

It is necessary to mention that concentrations of tested heavy metals are relatively high compared to the average values found in soils. The application of higher concentrations on plant roots in laboratory conditions allow for a fast recognition of the differences in plant sensitivity to particular contaminant. Therefore, this is a very important factor in research of plant tolerance mechanisms.

Table 1: Effect of lead (Pb), cadmium (Cd) and arsenic (As) on roots' length (in mm) in cereals (maize and barley)

Tabuľka 1: Vplyv olova (Pb), kadmia (Cd) a arzénu (As) na dĺžku koreňov (v mm) obilnín (kukurica a jačmeň)

Plant	Concentration of heavy metals (mg*L^{-1})	Day(s) after application of heavy metals with the corresponding IP values								
		0.	1st.	IP (%)	2nd.	IP (%)	3rd.	IP (%)	4th.	IP (%)
Barley	Control	2.40 ± 0.15	22.24 ± 0.64	–	46.02 ± 0.94	–	69.08 ± 1.52	–	86.68 ± 2.13	–
	Pb 300	2.51 ± 0.15	16.95 ± 0.53 *	23.79	30.69 ± 0.96 *	33.31	42.07 ± 1.23 *	39,01	50.60 ± 1.41 *	41.63
	Pb 500	2.39 ± 0.13	13.78 ± 0.60 *	38.04	22.11 ± 0.91 *	51.96	31.18 ± 1.29 *	54.86	37.47 ± 1.69 *	56.77
	Cd 100	2.55 ± 0.13	9.33 ± 0.52 *	57.15	13.13 ± 0.91 *	71.47	16.10 ± 0.93 *	76.69	17.11 ± 1.10 *	80.26
	Cd 300	2.55 ± 0.16	11.64 ± 0.75 *	47.66	12.91 ± 0.97 *	71.95	13.75 ± 1.01 *	80.09	13.87 ± 1.02 *	96.91
	As 50	2.30 ± 0.08	7.91 ± 0.57 *	64.43	8.53 ± 0.61 *	81.46	8.74 ± 0.66 *	87.35	8.81 ± 0.65 *	89.84
	As 100	2.40 ± 0.11	5.90 ± 0.31 *	73.47	6.06 ± 0.33 *	86.83	6.06 ± 0.33 *	91.23	6.06 ± 0.37 *	93.01
Maize	Control	4.04 ± 0.37	29.06 ± 1.86	–	64.29 ± 3.25	–	102.45 ± 4.80	–	128.04 ± 5.29	–
	Pb 300	3.51 ± 0.50	27.24 ± 1.29	6.26	58.04 ± 2.74 *	9.72	86.13 ± 3.63 *	15.93	104.93 ± 4.74 *	18.08
	Pb 500	3.45 ± 0.42	21.98 ± 1.23 *	24.36	46.67 ± 1.98 *	27.41	67.30 ± 2.76 *	34.31	80.47 ± 3.01 *	37.15
	Cd 100	4.20 ± 0.32	22.80 ± 1.21 *	21.54	42.67 ± 2.31 *	33.63	53.13 ± 2.71 *	48.14	58.70 ± 2.99 *	54.15
	Cd 300	4.96 ± 0.54	23.41 ± 0.96 *	19.44	24.27 ± 0.89 *	62.25	25.35 ± 1.28 *	75.26	25.35 ± 1.24 *	80.20
	As 50	4.40 ± 0.38	22.02 ± 0.69 *	24.23	28.16 ± 0.74 *	56.22	32.00 ± 1.05 *	68.77	33.18 ± 1.11 *	74.09
	As 100	3.92 ± 0.35	19.67 ± 0.89 *	32.31	25.61 ± 1.10 *	60.16	26.67 ± 1.13 *	73.97	26.96 ± 1.26 *	79.15

Data indicate ± standard deviation of mean values (n=45-60). Significant difference ($p < 0.05$) is denoted as asterisk (*) between control and heavy metal treatments. IP – index phytotoxicity.

Table 2: Effect of lead (Pb), cadmium (Cd) and arsenic (As) on roots' length (in mm) in legumes (bean cultivars)

Tabuľka 2: Vplyv olova (pb), kadmia (Cd) a arzénu (As) na dĺžku koreňov (v mm) strukovín (odrody bôbu)

Plant	Concentration of heavy metals ($mg*L^{-1}$)	Day(s) after application of heavy metals with the corresponding IP values								
		0.	1st.	IP (%)	2nd.	IP (%)	3rd.	IP (%)	4th.	IP (%)
Bean cv. Aštar	Control	5.22 ± 0.34	17.01 ± 0.81	–	30.02 ± 0.96	–	41.39 ± 1.24	–	47.60 ± 1.44	–
	Pb 300	5.42 ± 0.38	19.84 ± 0.70 *	-16.63	31.51 ± 1.17	-4.96	37.40 ± 1.61	9.64	39.85 ± 1.65	16.28
	Pb 500	5.43 ± 0.65	21.37 ± 1.43 *	-25.63	27.54 ± 1.40	8.26	29.69 ± 1.39 *	28.27	31.31 ± 1.38 *	34.22
	Cd 100	5.40 ± 0.45	18.93 ± 1.38	-11.29	21.53 ± 1.37 *	26.00	22.97 ± 1.42 *	44.50	23.27 ± 1.48 *	51.11
	Cd 300	5.60 ± 0.72	16.00 ± 1.09	5.94	17.53 ± 1.35 *	41.36	17.90 ± 1.26 *	56.75	18.08 ± 1.28 *	62.01
	As 50	5.14 ± 0.41	19.30 ± 0.66	-13.46	23.26 ± 0.85 *	22.52	25.40 ± 1.00 *	38.63	25.60 ± 1.04 *	46.22
	As 100	5.63 ± 0.39	13.67 ± 0.78 *	19.64	14.44 ± 0.79 *	51.90	14.50 ± 0.64 *	64.97	14.50 ± 0.04 *	69.53
Bean cv. Piešťanský	Control	5.13 ± 0.28	22.23 ± 1.88	–	36.69 ± 2.33	–	49.46 ± 2.90	–	56.67 ± 3.10	–
	Pb 300	5.63 ± 0.31	23.83 ± 1.02	-7.20	36.30 ± 1.04	1.07	43.07 ± 1.00	12,92	49.43 ± 1.10	12.77
	Pb 500	5.70 ± 1.03	17.70 ± 1.55	20.38	27.54 ± 1.61 *	24.94	33.27 ± 1.45 *	32.73	37.05 ± 1.49 *	34.62
	Cd 100	5.70 ± 0.25	22.10 ± 0.87	0.58	30.13 ± 1.10 *	17.88	34.15 ± 1.12 *	31.42	35.60 ± 1.21 *	37.18
	Cd 300	5.84 ± 0.34	19.22 ± 1.08	13.54	22.91 ± 1.09 *	37.56	23.22 ± 1.83 *	53.05	23.52 ± 1.21 *	58.50
	As 50	5.80 ± 0.24	22.23 ± 1.05	0.00	25.75 ± 0.92 *	29.82	26.73 ± 1.11 *	45.69	27.08 ± 1.15 *	52.21
	As 100	5.68 ± 0.29	17.55 ± 0.97 *	21.05	18.71 ± 0.96 *	49.00	18.97 ± 0.98 *	61.64	19.13 ± 0.93 *	66.24

Data indicate ± standard deviation of mean values (n=45-60). Significant difference ($p < 0.05$) is denoted as asterisk (*) between control and heavy metal treatments. IP – index phytotoxicity.

Table 3: Effect of lead (Pb), cadmium (Cd) and arsenic (As) on roots' length (in mm) in legumes (soybean and pea)

Tabuľka 3: Vplyv olova (pb), kadmia (Cd) a arzénu (As) na dĺžku koreňov (v mm) strukovín (hrach a sója)

Plant	Concentration of heavy metals (mg*L^{-1})	Day(s) after application heavy metals with the corresponding IP values							
		0.	1st.	IP (%)	2nd.	IP (%)	3rd.	IP (%)	4th.	IP (%)
Soybean	Control	6.39 ± 0.61	n.d.	–	n.d.	–	n.d.	–	94.28 ± 6.46	–
	Pb 300	6.40 ± 0.30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	60.07 ± 3.39 *	36.29
	Pb 500	5.95 ± 0.36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	43.90 ± 2.25 *	53.44
	Cd 100	6.11 ± 0.30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	33.00 ± 1.71 *	65.00
	Cd 300	6.33 ± 0.32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	37.17 ± 2.41 *	60.57
	As 50	5.77 ± 0.18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	18.57 ± 1.28 *	80.30
	As 100	5.77 ± 0.39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7.23 ± 0.25 *	92.33
Pea	Control	3.97 ± 0.21	n.d.	–	n.d.	–	n.d.	–	39.45 ± 2.07	–
	Pb 300	3.89 ± 0.19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29.20 ± 1.44 *	25.99
	Pb 500	4.03 ± 0.21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	27.28 ± 1.56 *	30.85
	Cd 100	4.32 ± 0.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	22.03 ± 1.59 *	44.16
	Cd 300	4.00 ± 0.19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11.31 ± 0.59 *	71.33
	As 50	4.26 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	18.18 ± 1.46 *	53.92
	As 100	4.27 ± 0.42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13.11 ± 0.71 *	66.77

Data indicate ± standard deviation of mean values (n=45-60). Significant difference ($p < 0.05$) is denoted as asterisk (*) between control and heavy metal treatments. IP – index phytotoxicity, n.d. – not determined.

Conclusion

In general, a toxic effect of the used heavy metals on root system was demonstrated in this work. Impact of the heavy metals on the plants' root system depended on the particular heavy metal, their concentration, plant species and overall period of exposure to the heavy metal. Lower effect of lead (IP up to 56.77 %) was observed in all variants of the experiment. IP increased over 50 % due to higher doses of cadmium and arsenic. While, in case of barley and maize, the toxic effect of almost all test doses of the metals was observed as soon as 24 hours after their applications, lower doses of applied metal solutions caused no changes or slow stimulation of root growth of tested bean cultivars. During the entire experiment, both varieties of bean showed, in general, higher tolerance to test metals (IP up to 69.53 %). The most sensitive reactions to the single doses of metals had roots of barley and soybean (IP > 50 %, excluding dose Pb 300). Out of the used set of heavy metals, the most toxic effect on the roots´ growth and their overall development had arsenic (As^{+3}). This element totally inhibited the roots´ elongation as well (IP > 80 % in case of barley and soybean).
Application of heavy metals at different concentrations and higher number of examined plants brings new knowledge about toxic effects of metals on plants. Moreover, this method can also be one of the ways how to discover plant´s resistance mechanisms.

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