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ORIGINAL ARTICLE

Monitoring the sensitivity of selected crops to lead, cadmium and arsenic

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Heavy metals are highly toxic environmental pollutants. In plants, these compounds cause numerous slighter or stronger toxic effects. They inhibit root and shoot growth and yield production, affect nutrient uptake and homeostasis, and are frequently accumulated by agriculturally important crops.

Effects of heavy metals on five selected species of agricultural crops were monitored. We focused our attention to general and commonly used stress indicators such as seed germination, weight and length of roots and shoots. Each of these characteristics was dependent on the tested plant species and tested heavy metals. Dosage of lead (500 mg/l) had little effect on seed germination, cadmium (300 mg/l) significantly affected seed germination of pea and barley, arsenic (100 mg/l) caused total inhibition of seed germination in all tested plant species. Plants grow in soil contaminated with heavy metals showed several symptoms of metal toxicity (chlorosis, necrosis of leaf tips, blackening of roots).

In general, the highest tolerance to tested metal ions was observed in both varieties of bean, and the lowest sensitivity was observed in soybean plants. The highest degree of toxicity was shown to have tested doses of cadmium and arsenic, the lowest the doses of lead. In general, the lowest tolerance indexes were determined based on the decrease in fresh weight of roots.

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Increasing environmental pollution caused by heavy metals, due to industrial and agricultural activities, is becoming a significant problem in the world. The excessive uptake of these metals from the soil can create dual problem: the harvested crops so contaminated represent a source of heavy metals in our food supply, and yields are reduced due to adverse effect on plant growth (Bala and Setia, 1990; Hall, 2002).

Toxic effects of some heavy metals on many agricultural crops were determined in various conections. There are significant differences in heavy metal tolerance among species and varieties, and contradictions exist between the results of different experiments (Nada et al., 2007).

High variability in plants' reaction to heavy metals, however, constantly brings new questions answers to which can play an important role with regard to maintaining the clean environment and health of the people. Application of several heavy metals in various concentrations on a higher number of examined plants not only brings new knowledge on toxic effects of metals on plants, but can also be one of the ways of searching for the discovery of plants' resistance mechanisms.

Examination of plants' sensitivity to heavy metal ions is carried out by various methods. Approaches based on determination of various growth characteristics (weight and length of roots and shoots, chlorophyll content) are still widely used and represent relatively cheap and quick methods of comparing plants' sensitivity to various stress factors as well as toxicity of single contaminants.

Aim of this study was to compare the effects of lead (Pb), cadmium (Cd) and arsenic (As) on seed germination and growth of five plant species (bean, pea, soybean, barley and maize) in early stage of ontogenesis.

MATERIAL AND METHODS

Germination assay

Seeds of barley (*Hordeum vulgare* cv. Garant), corn (*Zea mays* cv. Quintal), pea (*Pisum sativum* cv. Olivнn), soybean (*Glycine max* cv. Korada), beans (*Vicia faba* cvs. Aštar and Piešťanský) were surface-sterilized with 5% sodium hypochloride for 5 min to prevent fungal growth, then rinsed five times in distilled water. Afterwards the seeds of bean and maize were thoroughly rinsed with tap water and then soaked for 24 h in heavy metal solutions (500 mg/l Pb; 300 mg/l Cd and 100 mg/l As) at room temperature. The chemicals used in the tests were: $Pb(NO_3)_2$, $Cd(NO_3)_2.4H_2O$ and As_2O_3. Sterilized and soaked seeds were germinated in Petri dishes lined with two layers of water (control) and heavy metal moistened filter paper (Whatman No.1) in the dark at 25°C.

A number of germinated seeds ($n \geq 100$) were evaluated after 72 h. Seeds with roots longer than 1 mm were regarded as germinated in case of pea, barley and maize (Obroucheva et al., 1998) and roots longer than 3 mm were regarded as germinated in case of two varietas of bean. Total germination was expressed as percent of that in the control treatment.

Pot experiment

Sterilized seeds of tested plants were germinated in Petri dishes lined with two layers of water moistened filter paper (Whatman No.1) in the dark at 25°C. Uniformly germinated seeds were transplanted into pots (10 cm Ш; 250 ml containing mix of soil (BORA, pH 6-7, 1.0 % N; 0.3 % P_2O_5; 0.4 % K_2O) and perlite (4:1). The plants were cultivated in a growth chamber at 20ºC, 12 hours light/12 hours dark period, (illumination of 800 lux) and relative humidity 60-70%. Pots were watered daily to 60% water-holding capacity of the soil. When the first assimilating leaves were developed, plants were supplied with distilled water (control) or heavy metal solutions (500, 300 and 50 mg/kg soil of Pb, Cd and As respectively). The chemicals used in the tests were the same as in germination assay. Subsequently the plants were watered to maintain soil moisture at about 50% of water holding capacity by adding tap water during the experimental period. At day 10 of cultivation the plants were harvested and divided into shoots and roots to determine fresh weight. Shoot and root lengths were measured as well.

Three replicates were used per treatment and 5 plants per pot were analyzed. Metal tolerance was measured according to Wilkins (1978).

Data analysis

Experimental data were processed statistically by the Studentrs t-test. The Wilcoxon signed rank test was used for not normally distributed data.

RESULTS AND DISCUSSION

Effects of heavy metals on seed gemination

Seeds of the tested varieties showed differences in germination during cultivation in solutions of metal ions (Table 1). Out of the tested ions, the most toxic was As, which caused complete suppression of germination of seeds in case of all varieties. On the contrary, Pb did not cause significant changes in germination. Application of Cd resulted in statistically significant inhibition of germination of pea and barley seeds (by 29.37% and 64.76% respectively). Similar differences were also observed by other authors (Li et al., 2005), and the cause for the differences is considered to be a great interspecific variation in the morphology of seed coats (Abedin and Meharg, 2002; Di Salvatore et al. 2008). Hence, the permeability of the seed coat also depends upon the physical and chemical properties of different metals (Kochlar et al., 2004) and their concentration (Kranner and Colville, 2011). Luan et al (2008) e.g. showed, that seed germination was significantly influenced only when concentrations of Cd, Pb and As in the soil were relatively high (> 800 mg/kg) indicating some resistance of seed germination to toxicity of these metals. The seed germination does not seem to be a sensitive indicator of the phytotoxic effects of Cd, Pb and As in most experimental treatments (Kapustka et al., 1995; Luan et al., 2008).

Table 1. Percent germination of seeds exposed to Pb, Cd and As solution (mg/l).

Tested plant	Control	Pb 500	Cd 300	As 50
Bean cv. Aљtar	96,78 ± 1,70	91,52 ± 3,09	91,64 ± 3,50	n.d.
Bean cv. Piešťanský	94,40 ± 2,15	89,70 ± 3,84	87,60 ± 2,48	n.d.
Soybean	95,00 ± 1,64	95,00 ± 2,58	90,83 ± 2,39	n.d.
Pea	84,15 ± 3,33	80,89 ± 1,25	70,63 ± 2,37 **b**	n.d.
Barley	86,30 ± 1,16	85,56 ± 2,53	35,24 ± 4,36 **c**	n.d.
Maize	96,67 ± 2,11	96,67 ± 1,05	92,86 ± 2,11	n.d.

Data indicate ± standard deviation of mean values (n ≥ 100). Significance of differences: **b**) and **c**) for $P < 0.05$ and $P < 0.01$, respectively. n.d. – not determined.

Effect of heavy metals on growth of plants in contaminated soil

In the next phase of experiments, we tested sensitivity of selected crops to heavy metals applied to the soil in pots (500 mg Pb^{2+}/kg and 300 mg Cd^{2+}/kg of soil respectively) in the stage of forming first fully developed leaves. The used dose of As was reduced to 50 mg/kg of soil due to the high toxicity of the As dose applied to the seed during germination. Toxic effects of the metals were examined after 10 days of cultivating the plants in contaminated soil.

Plants' tolerance to metal ions was evaluated through the results of measurements of growth parameters: LR (length of root), LSH (length of shoot), WR (fresh weight of root) and WSH (fresh weight of shoot).

Roots responded to Cd and Pb more sensitively than shoots, while As proved to be about equally toxic to roots and shoots (Table 2). In general, out of all tested parameters, weight of roots was the most and length of shoots the least sensitive parameter, so in the further comparison, the tolerance was expressed by weight of roots. On the basis of TI calculated from this parameter, we created the following order of the tested plants' sensitivity to metal ions:

a) Pb: bean cv. Aštar > bean cv. Piešťanský > soybean > maize > barley > pea,

b) Cd: bean cv. Aštar > bean cv. Piešťanský > soybean > maize > barley > pea,

c) As: bean cv. Piešťanský = pea > maize = bean cv. Aštar > barley > soybean.

To As reacted most sensitively soybean, which died as early as 24 hours after application of metal. Out of tested metal ions, Pb showed the lowest toxicity (TI = 68–100), while As the highest toxicity (TI= 20–57) in the group of tested plants.

Generally low or moderate toxicity of Pb to various plant species including faba bean, maize and pea was reported also by other authors (Tung and Temple, 1996; Piechalak et al., 2002). Berry (1924) considered adding Pb (in the form of nitrate) to soil in certain concentration a potentially better fertilizer than sodium nitrate and more beneficial than harmful. Roots reacted to Cd and Pb more sensitively than shoots, and under the effect of As, a significant reduction in growth of roots as well as shoots occurred. Maize and pea showed higher degree of tolerance to As than to Pb or Cd. According to Evans et al. (2005), As in low doses (100 µg/l) can be even beneficial to growth and development of maize. Physiological and biochemical causes for this phenomenon have not been completely clarified yet. Growth stimulation by As can be caused by displacement of phosphate ions from the soil by arsenate ions, with the resultant increase of phosphate availability (Jacobs and Keeney, 1970).

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 even between genotypes of the same species (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).

In cases of soybean and both faba-bean varieties, we observed in some parameters stimulatory effect of the tested dose of metal (TI > 100). The observed stimulatory effect, appearing after application of metal solution, is probably the result of the effect of NO^{3-} ions from the applied solution or the dose of the metal itself (Yogeetha et al., 2004; Bashmakov et al., 2005). However, the existence of the phenomenon of 'hormesis' (states that many non-essential chemicals stimulate plant growth and other biological processes at low doses, but inhibit such processes at higher levels, Jäger and Krupa, 2009) cannot be confirmed or excluded through our experiments.

Table 2. Growth parameters of tested plants grown in soil saturated with Pb, Cd, As and water (control).

	Concentration of heavy metals (mg/kg soil)						
Plant	**0 (control)**	**Pb 500**	**TI (%)**	**Cd 300**	**TI (%)**	**As 50**	**TI (%)**
Length of root (cm)							
BA	19,18 ± 0,81	18,17 ± 2,84	94,73	17,49 ± 1,07	91,19	11,83 ± 0,64 **c**	61,68
BP	17,83 ± 0,76	17,90 ± 1,02	100,39	16,92 ± 0,65	94,90	10,60 ± 0,68 **c**	59,45
S	31,98 ± 3,10	26,00 ± 0,86	81,30	27,18 ± 2,66	84,99	n.d.	
P	12,50 ± 0,82	12,10 ± 1,10	96,80	12,30 ± 0,97	98,40	7,50 ± 0,74 **b**	60,00
B	33,45 ± 1,47	34,36 ± 2,03	102,72	19,43 ± 2,21 c	58,09	9,50 ± 2,16 **c**	28,40
M	37,88 ± 0,15	37,75 ± 0,73	99,66	34,80 ± 2,20	91,87	33,66 ± 5,50	88,86
Length of shoot (cm)							
BA	16,59 ± 0,81	18,40 ± 1,16	110,91	15,77 ± 0,30	95,06	11,25 ± 1,38 **b**	67,81
BP	23,96 ± 0,60	24,32 ± 0,98	101,50	25,90 ± 1,20	108,10	16,30 ± 1,20 **c**	68,03
S	21,76 ± 1,16	24,16 ± 0,58	111,03	20,87 ± 0,91	95,91	n.d.	
P	13,42 ± 0,82	11,40 ± 0,94	84,95	9,70 ± 0,77 **b**	72,28	8,30 ± 1,31 **b**	61,85
B	35,35 ± 0,68	34,11 ± 1,64	96,49	31,11 ± 2,71	88,00	12,98 ± 1,70 **c**	36,72
M	32,77 ± 1,08	31,47 ± 0,55	96,03	28,81 ± 1,73	87,92	26,70 ± 3,36	81,48
Fresh weigth of root (g)							
BA	2,34 ± 0,18	2,34 ± 0,25	100,00	2,56 ± 0,23	109,40	1,02 ± 0,18	43,59
BP	2,90 ± 0,54	2,85 ± 0,21	98,28	2,40 ± 0,46	82,76	1,64 ± 0,06 **a**	56,55
S	0,52 ± 0,05	0,39 ± 0,02 **a**	75,00	0,40 ± 0,03 **a**	76,92	n.d.	
P	0,50 ± 0,04	0,34 ± 0,05 **a**	68,00	0,32 ± 0,06 **a**	64,00	0,28 ± 0,08 **a**	56,00
B	0,15 ± 0,01	0,13 ± 0,02	86,67	0,10 ± 0,01 **a**	66,67	0,03 ± 0,01 **c**	20,00
M	1,07 ± 0,10	0,99 ± 0,04	92,52	0,74 ± 0,08 **a**	69,16	0,47 ± 0,13 **b**	43,93
Fresh weigth of shoot (g)							
BA	2,92 ± 0,17	2,77 ± 0,21	94,86	2,81 ± 0,21	96,23	2,53 ± 0,19	86,64
BP	7,03 ± 0,73	7,35 ± 0,13	104,55	6,11 ± 0,28	86,91	4,37 ± 0,48 **b**	62,16
S	1,52 ± 0,09	1,52 ± 0,07	100,00	1,42 ± 0,15	93,42	n.d.	
P	0,92 ± 0,10	0,70 ± 0,12	76,09	0,62 ± 0,08 **a**	67,39	0,54 ± 0,12 **a**	58,70
B	0,81 ± 0,07	0,80 ± 0,10	98,77	0,57 ± 0,08 **a**	70,37	0,15 ± 0,03 **c**	18,52
M	1,96 ± 0,15	1,91 ± 0,06	97,45	1,52 ± 0,16	77,55	1,30 ± 0,20 **a**	66,33

Data indicate ± standard deviation of mean values (n = 15). Significance of differences: **a**), **b**) and **c**) for $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. TI – tolerance index, BA – bean cv. Aštar, BP – bean cv. Piešťanský, S – soybean, P – pea, B – barley, M – maize, n.d. – not determined.

Evidence of metal toxicity to plants was present especially in variant of the Cd and As polluted soil. The visual phytotoxicity symptoms observed were slight chlorosis, necrosis of leaf tips in case of barley and pea treated with As, blackening of roots and basal parts of shoots of beans treated with Cd and As. Blackening was appearing on faba bean roots which can indicate metal-induced oxidation of different phenols in roots (Fecht-Christoffers et al., 2003). Except in cases of bean and pea, slight chlorosis was observed on leaves of plants affected by Cd and As. It has been suggested that the origin of chlorosis lies in the roots; the plants are unable to utilize iron, resulting in an iron-deficiency chlorosis common to all metals (Hewitt, 1948).

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