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EFFECT OF CADMIUM ON GROWTH, PHOTOSYNTHETIC PIGMENTS, IRON AND CADMIUM ACCUMULATION OF FABA BEAN (*VICIA FABA* CV. AŠTAR)

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The influence of different concentrations of cadmium (Cd) ions (50 and 100 mg/kg soil) on growth, photosynthetic pigment content, Cd, and iron accumulation in faba bean (*Vicia faba* L. cv. Aštar) was studied under laboratory conditions. No significant changes were observed in the growth parameters of shoots (length, fresh, and dry weight). Both tested Cd doses resulted in decrease in root fresh weight by 31.7% and 28.68% and in dry weight by 32.2% and 33.33%, respectively. Increased accumulation of Cd was observed in roots (125- and 173- fold higher than in control) and shoots (125- and 150- fold higher than in control) as a result of applied doses of Cd. Increased accumulation of iron was detected in roots (1.45- and 1.69-fold higher than in control). Decrease in the content of chlorophyll *a* (by 25.52 and 24.83%, respectively) and chlorophyll *b* (by 6.90%) after application of Cd 100 as well as decrease in carotenoids (by 40.39 and 38.36%, respectively) was detected. Weak translocation of Cd from roots to shoots pointed to low phytoremediation potential of the tested bean variety in contaminated soil. However, the high tolerance of this cultivar, its relative fast growth, as well as priority of Cd accumulation in roots presume this plant species for phytostabilisation and revegetation of the Cd-contaminated soils.

Key words: faba bean, cadmium, tolerance, photosynthesis, oxidative stress, remediatory potential

Contamination of soils with Cadmium (Cd) is a major threat to ecosystems. Cd is rapidly taken up by plant roots and can be loaded into the xylem for its transport to leaves. Many species accumulate toxic metals mainly in the roots (Benavides *et al*. 2005); according to Wu (1990), about 70–85% of the absorbed Cd remains in the roots in various plants. The differences in Cd accumulation capacity and localisation appear to be the major factors in determining plant tolerance to Cd exposure (Obata & Umebayashi 1993). The toxic effect of Cd is related to its ability to generate reactive oxygen species (ROS) resulting in unbalanced cellular redox homeostasis (Schützendübel *et al*. 2001). The ROS generation is indirect because Cd does not participate in Fenton-type reactions; therefore, it is a non-redox metal (Romero-Puertas *et al*. 2004). In plants, exposure to Cd causes inhibition of growth, activation or inhibition of enzymes, reduction of transpiration rate and water content (Benavides *et al.* 2005). Stomatal closure due to entry of Cd into the guard cells in competition to Ca^{+2} (Perfus-Barbeoch

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et al. 2002) and reduction in stomata count per unit area are also characteristic symptoms of Cd stress resulting in lesser conductance to CO_2 (Pietrini *et al*. 2010), which consequently lead to the overall inhibition of photosynthesis. In addition, Cd may disturb plant mineral metabolism. For example, Cd almost completely inhibits iron (Fe) translocation from roots to shoots, leading to increased root Fe concentrations in plants (Muradoglu *et al.* 2015).

Many studies have attempted to clarify the mechanism of Cd toxicity in plants (Békésiová *et al.* 2008; Tamás *et al.* 2012; Balestri *et al.* 2014); however, relationships between growth inhibition and physiological processes under Cd condition are still discussed. Mainly because of the fact that its toxic effects are expressed in relation to plant species or varieties. The toxicity of Cd is also greatly influenced by the concentration of Cd^{2+} ions, their form and availability in the soil, duration of their application, as well as by other different factors of the environment (pH of the soil, soil humidity, and others). There are also no univocal reports on the relationships between Cd stress and some physiological processes (e.g., water relations) since Cd can interfere in several ways on the parameters that affect these physiological processes in leaves (Barceló & Poschenrieder 1990). Knowledge of mechanisms of plants' tolerance to heavy metals ions provides an opportunity of breeding varieties suitable for phytoremediation. Besides, metal hyper-accumulating plants, non-accumulating Cd, and high biomass crops are also considered for phytoextraction purposes, but it has been suggested that the success of this approach might be limited by Cd-induced phytotoxicity problems (McGrath *et al.* 2001). Although plants belonging to family *Fabaceae* are sensitive to high concentrations of heavy metals (Kuboi *et al.* 1987), several studies indicated that plant such as *Lupinus albus or Vicia faba* are used in re-vegetation and phytostabilization of cadmium contaminated soils (Vazquez *et al*. 2006; Pichtel & Bradway 2008).

In the presented article, the influence of different concentrations of Cd ions (50 and 100 mg/kg soil) on growth, photosynthetic pigment content, Cd and Fe accumulation in faba bean (cv. Aštar) is presented. In addition, the potential of broad bean for the phytoremediation of Cd in a contaminated soil was presented.

MATERIAL AND METHODS

Plant material and growth conditions

Seeds of beans (*Vicia faba* cv. Aštar) were surface-sterilized with 5% sodium hypochloride for 15 min and planted in pots 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 20°C, 12 h light/12 h dark period (illumination of 400 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 two doses of Cd: 50 (Cd 50) and 100 (Cd 100) mg/kg of soil, respectively. Cd was added as $Cd(NO_3)_2.4H_2O$.

The test concentrations of cadmium were used due to predicted toxicity of this element to bean plants (Piršelová *et al*. 2015).

Growth parameters

On day 10 after application of metal solutions (BBCH 31-2 visibly extended internodes), roots were separated from the above-ground part of the plants, washed with tap water, and growth parameters (length and fresh weights) were determined. After washing, the plant samples were oven-dried at 70°C for 24 h to constant dry weight, and this parameter was also determined. Three replicates were used per treatment and eight plants from each pot were analysed (altogether 24 plants).

Photosynthetic pigments determination

For photosynthetic pigments (chlorophyll *a* and *b*, carotenoids) analysis, fully developed trifoliate leaves were extracted with 80% acetone. Pigments contents were determined spectrophotometrically (UV-VIS spectrophotometer, Shimadzu) at the following wavelengths: 663, 646 and 470 nm and calculated according to Lichtenthaler and Wellburn (1983). The experiment was performed in four replicates.

Determination of tolerance index

Tolerance index (TI) was calculated as a ratio of the mean dry weight of plants grown in the presence of Cd and the mean dry weight of control plants expressed as percentage.

In vivo detection of H_2O_2 in leaves

Diaminobenzidine (DAB) was used for the detection of H_2O_2 staining in leaf tissues (Thordal-Christensen *et al.* 1997). On day 10 after application of metal solutions, fully developed leaves (the first bifoliate – developmental stage 1 and second trifoliate – developmental stage 2) excised from Cd-treated plants (50 and 100 mg Cd^{2+}/kg soil) or from untreated plants were placed in Petri dishes containing DAB solution (1 mg/ml). Plates were left in a climate chamber at 24°C in darkness, and DAB staining was assessed visually 12 h later. Leaves were bleached by immersing in boiling ethanol to visualize the brown spots characteristic of the reaction of DAB with H_2O_2.

Measurements of metal content in leaves and roots

Dried plant material (0.5 g roots and shoots) was digested in the mixture of 5 ml water, 5 ml of concentrated HNO_3 p.a. (Merck, Darmstadt, Germany), and 1.5 ml of H_2O_2 p.a. (Slavus, Bratislava) by using the microwave oven Mars Xpress (CEM Corporation, Matthews, USA). Decomposition temperature was 140°C, ramp time 15 min, and hold time 13 min. After digestion, the solution was diluted to 25 ml with deionised water and filtered through an acid-resistant cellulose filter (Whatman No. 42). Blank samples were prepared in a similar way. The elements (Cd and Fe) were determined by electrothermal atomic absorption spectroscopy (AAS Perkin Elmer 1100B, Norwalk, Connecticut, USA).

The biological accumulation coefficient for cadmium - BAC, biological transfer coefficient - BTC and biological concentration factor - BCF were determined (Tukura *et al.* 2012).

BAC = (metal content in the above-ground part of plant/metal content in soil) × 100

BTC = (metal content in the above-ground part of plant/metal content in root) × 100

BCF = (metal content in root/metal content in soil) × 100

Statistical analysis

Data were analysed by one-way ANOVA or Kruskal-Wallis tests using XLSTAT software. The significance of differences between the concentrations of heavy metals in plant tissues was shown by using the Student's t-test, $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Plant growth

Growing in a contaminated soil, the bean plants did not show any apparent visual symptoms of intoxication by the metal. Similar conclusion was also reached by Dobroviczká *et al*. (2013) at cultivation of soybean (*Glycine max* cv. Bólyi 44, cv. Cordoba) in soil contaminated with Cd in concentration of 50 mg/kg soil and by Pinto *et al.* (2004), who exposed sorghum (*Sorghum* sp.) to various doses of Cd.

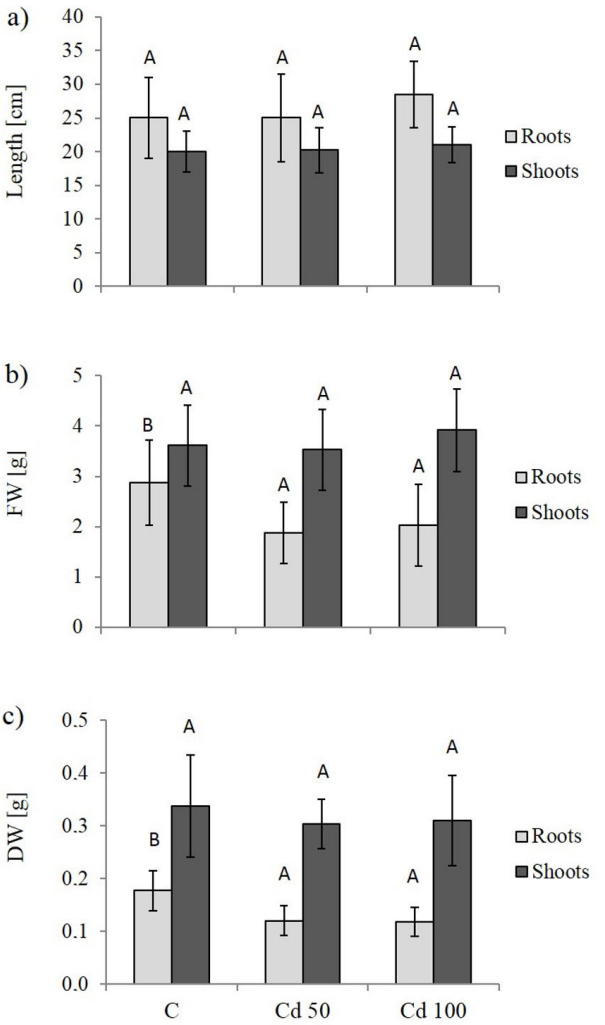

Figure 1. Effect of cadmium on length – a, fresh weight (FW) – b, and dry weight (DW) – c of roots and shoots of bean plants. Data are presented as means ± SD, n = 24. Different letters indicate significant differences at $p < 0.05$.

Plant length, fresh and dry weight of shoots were not significantly affected by Cd (Figure 1); however, each of the tested doses of Cd resulted in decrease of root fresh weight by 31.70 and 28.68% and dry weight by 32.2% (TI = 67.80) and 33.33% (TI = 66.67), respectively (Figure 1). Decrease in root biomass after exposure to Cd was also observed by others (Kochlar *et al*. 2004; Rodriguez-Serrano *et al.* 2009). By contrast, low doses of Cd often cause increase in the amount of fresh biomass of shoots (Pinto *et al.* 2004; Shah *et al*. 2008). In our experiment, due to doses Cd 50 and Cd 100, the length of shoots was also increased by 1.35% and 5.08% (Figure 1a), and fresh biomass of shoots was increased by 0.82 and 4.41%, respectively (Figure 1b). Detected TI calculated on the dry mass of roots and shoots (66.67–91.99) suggests high tolerance of the given variety to Cd. Plants with TI higher than 60 are considered as tolerant (Lux *et al.* 2004).

Accumulation of Cd and Fe in plant tissue

With increased concentration of the applied metal, also the increased accumulation of Cd in roots (125 and 173-more compared to the control) and in shoots (125 and 150-more compared to the control) of faba bean was observed (Table 1). Our results indicate that the majority of Cd was accumulated in the roots, which suggests a strong Cd retention during its long distance transport from roots to shoots, which might be a plant mechanism to tolerate the metal stress (Zornoza *et al.* 2002). Increased Fe accumulation was detected only in roots (1.5 and 1.69-more compared to control). In shoots, just the same content of Fe was detected in control as well as in stressed samples (Table 1). Our results correspond to the results of Luo *et al.* (2012), who observed increased accumulation of given metal and Fe mainly in roots influenced by Cd concentration. The intake of Fe from the soil by roots in non-graminaceous monocots and dicots is primarily regulated by the Fe transporter IRT1 (Curie & Briat 2003). Several studies also provide strong evidence that the Fe transporter IRT1 is also primarily responsible for Cd^{2+} influx into root cells (Vert *et al.* 2002).

Although no leaves chlorosis and no changed Fe content in shoots were observed in our experiments, strong differences in the Fe content in roots and shoots indicate inhibition of Fe translocation from roots to shoots. Although the mechanism underlying Cd-induced Fe deficiency in plants has not been identified, there are several possible explanations. The root Fe-deficiency-inducible enzyme Fe(III)-chelate reductase is inhibited by Cd (Parmar

T a b l e 1

Cadmium (Cd) and iron (Fe) content in roots and shoots [μg/g dry weight]

Variant of experiment	Root		Shoot	
	Cd	Fe	Cd	Fe
Control	0.50 ± 0.01	1,035 ± 103.00	0.11 ± 0.03	117 ± 1.53
Cd 50	62.26 ± 9.60^{+}	1,503 ± 175.00^{+}	13.73 ± 3.27^{+}	108 ± 0.71
Cd 100	86.40 ± 0.99^{+}	1,754 ± 104.00^{+}	16.53 ± 4.37^{+}	119 ± 11.72

Data are presented as means ± SD; n = 3; $^{+}$indicate the level of significance at $p < 0.05$

T a b l e 2

Effect of soil pollution with cadmium on the biological accumulation coefficient (BAC), biological transfer coefficient (BTC), and biological concentration factor (BCF)

Variant of experiment	BAC	BTC	BCF
Cd 50	0.275	0.221	1.245
Cd 100	0.165	0.191	0.864

et al. 2013), suggesting that Cd may directly impair Fe acquisition. Also, Cd usually accumulating in roots, almost completely inhibits Fe translocation from roots to shoots, leading to increased root Fe concentrations in strawberry (Muradoglu *et al.* 2015) and mung bean (Liu *et al.* 2000).

As a result of the Cd accumulation in roots, the BAC and BTC values were very low and less than 1 (Table 2). Despite the relative high value of BCF at lower concentration of Cd (BCF > 1) was determined, bean are not suitable for phytoremediation of soils contaminated with Cd because of low BAC and BTC values. Plants exhibiting BTC (particularly BCF) value less than one are unsuitable for phytoextraction (Fitz & Wenzel 2002). However, higher BCF values (Table 2) presume this plant species for phytostabilisation and revegetation of the Cd-contaminated soils. By the influence of higher doses of Cd, low decrease in values of BAC, BCF, and BTC were observed (Table 2), probably as an effect of Cd toxicity. Cd-dependent increase of BTC at lower concentration and decrease at higher concentration of Cd were also observed by de Maria *et al.* (2013) in sunflower.

Pigment content and H_2O_2 accumulation in leaves

Upon the exposure to both doses of Cd, decreases in content of chlorophyll *a* (by 25.52% and 24.83%, respectively), chlorophyll *b* (by 6.90% upon application of Cd 100 only) as well as carotenoids (by 40.39% and 38.36%, respectively) were detected (Figure 2). These decreases were statistically significant. Reduction of the pigment contents in our study is comparable with the results of Kumar *et al*. (2000), who observed reduction of chlorophyll *a* by 38.37%, chlorophyll *b* by 26.27% and carotenoids by 31.27% in broad bean leaves treated with Cd (120 mg/kg soil).

The results of the effect of Cd on the ratio of chlorophyll *a* and *b* diverge. The results of many authors suggest that Cd ions cause degradation of chlorophyll *a* more rapidly than chlorophyll *b*, resulting in decreased Chl *a*/*b* ratio (Myśliwa-Kurdziel & Strzałka 2002; Kummerová *et al.* 2010). On the contrary, increased Chl *a*/*b* ratio was observed by some authors (Azevedo *et al*. 2005). From the data available in the literature, it is difficult to conclude to what extent the changes in the Chl *a/b* ratio

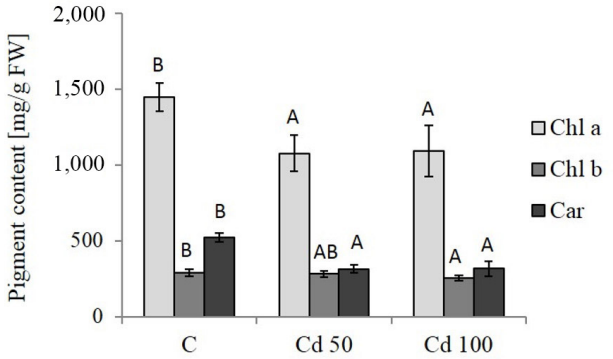

Figure 2. Chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), and carotenoids (Car) contents in leaves affected by Cd (50 or 100mg/kg soil). Data are presented as means ± SD, n = 4. FW – fresh weight. Different letters indicate significant differences at $p < 0.05$.

caused by metal stress are the result of the inhibition of the enzymatic activity converting Chl *a* to Chl *b* and to what extent they derive from different rate of degradation of both chlorophyll species (Myśliwa-Kurdziel & Strzałka 2002). Carotenoid content in plants exposed to Cd also does not exhibit a set pattern, and may either increase or decrease. The increase was observed in *Cucumis sativus* (Burzynski & Zurek 2007) and *Nicotiana tabacum* (Procházková *et al.* 2014). Oppositely, decrease was also observed, for example, in *Pisum sativum* (Hattab *et al.* 2009).

Inhibitory effect of Cd on photosynthetic apparatus has previously been reported by many other authors (Kummerová *et al.* 2010; Wang *et al*. 2013), although the opposite reaction has also been observed (Bindhu & Bera 2001). Reduction of chlorophyll content could result in enzymatic degradation of these pigments or inhibition of their biosynthesis, which could be connected with Cd-induced deficiency of Fe and zinc, decrease of magnesium content or Cd bond to essential thiol groups in various enzymes (Parmar *et al.* 2013). Cd does not participate in Fenton-type reactions; therefore, it can only indirectly lead to oxidative stress (Romero-Puertas *et al.* 2004). Thus, it is much more likely that Cd-related oxidative stress is a consequence of inhibition of photosynthesis, especially in leaves. This fact is supported by the results of histochemical staining of bean leaves with DAB for detection of H_2O_2 (Figure 3).

Despite the fact that on leaves no symptoms of toxicity have been observed, Cd induced a significant accumulation of H_2O_2 especially in older bean leaves treated with higher dose of Cd (Figure 3). While the content of Cd was not examined in different developmental stages of leaves, higher accumulation of H_2O_2 was observed in older leaves, which may indicate increased accumulation of Cd in older leaves compared with younger. The high Cd concentration, found mainly in roots and old leaves, suggests that plants tend to avoid toxicity in the physiologically most active portions of the plants by reducing Cd translocation to the epigeous portion, and by promoting the re-translocation of toxic metals from shoots to roots (de Maria *et al.* 2013).

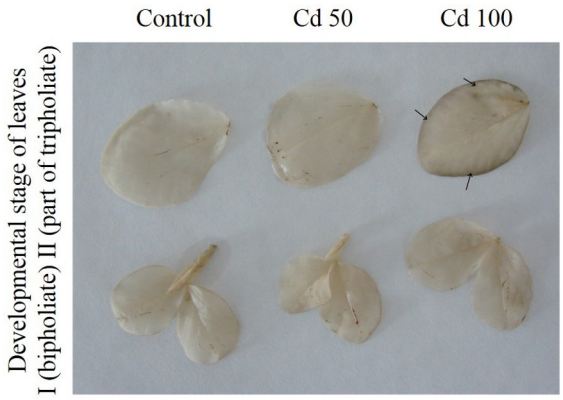

Figure 3. Histochemical detection of H_2O_2 in faba bean leaves. Arrows indicate brown deposits of H_2O_2.

CONCLUSIONS

The tested concentrations of cadmium (Cd) resulted in no visible symptoms of toxicity on faba bean cv. Aštar. Our results clearly demonstrated that photosynthetic apparatus of faba bean responded sensitively to the tested doses of Cd despite the high tolerance of the tested cultivar (TI > 60); however, disruption of photosynthetic apparatus is probably not the direct effect of Fe deficiency in shoots, but by Cd-induced changes in content of active iron (Fe) in cells (Luo *et al.* 2012) by emergent oxidative stress or other mechanisms. Low values of BAC and BTC show low phytoremediation potential of the given plant species in contaminated soils; however, the high tolerance of this cultivar, its relative fast growth, high biomass as well as priority of Cd accumulation in roots presume this plant species for phytostabilisation and revegetation of the Cd-contaminated soils. More in-depth biochemical and molecular biological analyses can contribute to revealing some further potential mechanisms of resistance of this faba bean variety to Cd.

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