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# EFFECTS OF CADMIUM AND ARSENIC IONS ON CONTENT OF PHOTOSYNTHETIC PIGMENTS IN THE LEAVES OF *GLYCINE MAX* (L.) MERRILL

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**Abstract**

Heavy metals belong to significant pollutants of environment accumulate in organisms and are unable to degrade. The goal of our experiments was to measure spectroscopically the content of photosynthetic pigments in the leaves of two soybean cultivars (*Glycine* max L.) exposed to cadmium (50 $mg.kg^{-1}$ of soil $Cd^{2+}$) and the metalloid arsenic (5 $mg.kg^{-1}$ of soil $As^{3+}$). Electrothermal atomic absorption spectroscopy analyses showed that after 10 days of plant growth in contaminated soil the shoots of the cv. Bólyi 44 accumulated much more Cd than As, and in both cases in significantly larger amounts than in the cv. Cordoba. However, leaves of the cv. Cordoba exerted more signs of intoxication since statistically significant decrease of some chlorophyll (Chl) content such as Chl*a*, Chl(*a*+*b*) and Chl*a*/*b* were detected in the $Cd^{2+}$-treated leaves. Our results revealed, that leaves of the cv. Bólyi 44 has significantly higher amount of carotenoids that might serve as protection compounds against metal damage.

## Introduction

The metalloid arsenic and the heavy metal cadmium are found naturally at low concentrations in the earth´s crust, while main natural sources of these metals are volcanic eruptions. However, they are also released to the environment as a result of different anthropogenic activities (Kazantzis, 1987; Rühling, 1994). As a consequence, leaking of these metals into atmosphere, soil or into irrigation water is becoming a serious environmental issue since can affect the normal growth and development of plants, and after entering the food chain represents a health risk for animals and humans as well.

Both arsenic and cadmium (for simplicity henceforth used for both the term "metal") are considered to be biologically nonessential. However, being neighbouring elements of phosphorus and zinc (respectively) in the respective columns of the periodic table, they tend to substitute for P and Zn in the cellular metabolism. This renders them potentially toxic for the living cells. Cellular As(V), is usually rapidly reduced to As(III), which behaves as a sulphur-seeking heavy metal ion, rather like $Cd^{2+}$ (Verbruggen *et al*., 2009). Therefore, there is a degree of similarity between the toxicologies of, and the sequestration machineries of the two metals. Toxic effects of arsenic and cadmium on the seedlings of crops has been reported by several authors (Abedin & Meharg, 2002; Liu *et al*., 2005; Li *et al*., 2007), while the symptoms of metal toxicity in plants were manifested by wilting, chlorosis and dying of the leaves (Stoeva *et al*., 2005; Bavi *et al*., 2011), limiting of the growth of roots and shoots (Carbonell-Barrachina *et al*., 1998; Shafi *et al*., 2010; Mészáros *et al*., 2013), decreasing of the length of roots and shoots (Stoeva *et al*., 2005; Shri *et al*., 2009; Ozdener & Kutbay, 2011) and decreasing the yield of fruits and grains (Rahman *et al*., 2007).

Leaf pigment content provides valuable information about the physiological status of plants and can indicate to occurring metal intoxication. The chlorophylls (Chls) are essential for the conversion of light energy to stored chemical energy, while their content can directly determine the photosynthetic potential and primary production (Curran *et al*., 1990). In addition Chls indirectly indicate to nutrition status of plants since much of leaf nitrogen is incorporated in chlorophyll (Filella *et al*., 1995; Moran *et al*., 2000). Furthermore, leaf Chl content is closely related to plant stress and senescence (Hendry *et al*., 1987; Merzlyak & Gitelson 1995; Merzlyak *et al*., 1999). On the other hand, another type of pigments – the carotenoids (Car) – play a structural role in the organization of photosynthetic membranes, participation in light harvesting, energy transfer, interception of deleterious free oxygen and organic radicals and quenching (Young & Britton, 1990).

Since heavy metals can affect each photosynthetic component at different level creating changes in some part of plants physiology and not in others (Li *et al*., 2009), the goal of this study was to assess the accumulation and effect of both cadmium and arsenic (respectively) on contents of basic photosynthetic pigments in two soybean cultivars. Though in plants the metals accumulate mainly in the root system, soybeans were shown to have higher concentrations of metal in the edible portion comparing to other crops (Sugiyama *et al*., 2011). Therefore, we focused on metal impact on the shoot system of the tested soybean plants. Studies on metal translocation to aboveground organs are expected to provide further knowledge for control of heavy metal pollution and food safety.

## Materials and Methods

**Plant growth condition and exposure to metals:** Surface sterilized seeds of soybean (*Glycine max* L. Merrill. cv. Bólyi 44, cv. Cordoba) were germinated in the Petri dish (Ø 15 cm) for 3 days. When the roots

reached a length of 5-8 mm, they were planted to pots (Ø 25 cm, volume 1 500 ml) to a mixture of peat soil BORA (pH 5 to 7, humidity 65%) and perlite (in a ratio of 4:1). Plants were grown until the unifoliolate (UL) and the first trifoliate leaves (TL) had fully developed, and the second trifoliate leaf was half-developed. They were flooded with distilled water, amount of which corresponded to the maximum sorption capacity of soil (~700 ml). The dose of metal (5 $mg.kg^{-1}$ of soil $As^{3+}$ and 50 $mg.kg^{-1}$ of soil $Cd^{2+}$) in the form $As_2O_3$ and $Cd(NO_3)_2.4H_2O$ solution (respectively) and distilled water as control were applied on plants on the $10^{th}$ day of experiment. The experiments were conducted in a growth chamber with a controlled climate at 23°C. After 10 days of plant growth in control and contaminated soil, the growth parameters such as shoot length, fresh weight and dry weight were determined.

**Measurements of pigment content in leaves:** Chlorophyll (Chl) and carotenoid (Car) content was determined spectrophotometrically (UV-VIS spectrophotometer, *Shimadzu*, Tokyo, Japan) at wavelengths 663 nm (Chl*a*), 645 nm (Chl*b*), 470 nm (Car), and calculated according to Lichtenthaler & Wellburn (1985). For each treatment, measurements were carried out on 8-10 leaves that were taken from 5 plants. The experiment was performed in four replicates.

**Measurements of metal content in leaves:** Dried plant material (0.5g) was digested in the mixture of 5ml water, 5ml of concentrated $HNO_3$ p.a. (Merck, Darmstadt, Germany) and 1.5ml of $H_2O_2$ p.a. (Slavus, Bratislava, Slovakia) by using the microwave oven Mars Xpress (CEM Corporation, Matthews, USA). Decomposition temperature was 140°C, ramp time 15 minutes and hold time 13 minutes. After digestion the solution was diluted to 25 ml with deionized water and filtered through an acid-resistant cellulose filter (Whatman No. 42). Blank samples were prepared in a similar way. The elements (As and Cd) were determined by electrothermal atomic absorption spectroscopy (AAS Perkin Elmer 1100B, Norwalk, Connecticut, USA) equipped with deuterium background correction and HGA 700 graphite furnace with automated sampler AS-70. Arsenic content was measured at wavelength 189 nm, slit 2 nm, and lamp current EDL 8W. Working conditions for Cd were: wavelength 228.8 nm; slit 0.2 nm and lamp current EDL 4.5W.

To prepare calibration solutions, aliquots were taken from a stock standard solution 1000 $mg.l^{-1}$ of As/Cd (Merck, Darmstadt, Germany). The calibration range was 5 - 20 $\mu g.l^{-1}$. The matrix modifier (Ru) was used by the determination of As. The accuracy of analytical results for As/Cd content in plant samples was checked by the analysis of the certified reference material BCR No 60 (trace elements in an aquatic plant). The recommended value for As was 8 $\mu g.g^{-1}$ and the determined value was 8.1$\mu g\ g^{-1}$; for Cd the certified value was 2.2± 0.1$\mu g.g^{-1}$ , the determined value was 2.2 ± 0.2$\mu g.\ g^{-1}$.

**Statistical analyses:** Each experiment was performed at least in triplicate. For statistical analyses the software JMP IN 8.0.2. was used. After verification of normal distribution and variance homogeneity the data was analysed by t-test (comparison of two treatments) or ANOVA with subsequent student's t-test or Tukey-test with $\alpha = 0.05$. If normal distribution was not fulfilled nonparametric tests (Wilcoxon) were applied. Data in the tables are expressed as the means of replicates ± standard deviation (SD).

**Results and discussion**

The doses of metal (loid) applied in this work are comparable or higher than are commonly found in soils in Europe; the mean value for As content in soils of Slovakia is 7.2 $mg.kg^{-1}$ of soil (Dragun, 1998) and for cadmium is 1.24 $mg.kg^{-1}$ of soil (Hronec, 1996). Different literature data indicate that in different plant species (Peralta *et al*., 2001; Stoeva *et al*., 2005; Ahmad *et al*., 2012) and lichens and moss (Bačkor *et al*., 2010; Pisani *et al*., 2011) they caused different toxicity symptoms. For example, Peralta *et al*., (2001) reported a reduction of shoot length of *Medicago sativa* at the applied dose of 5 mg and 20 mg $Cd^{2+}.l^{-1}$, while the dose of 40 mg $Cd^{2+}.l^{-1}$ was lethal. Stoeva *et al*., (2005) reported that immersion of bean roots in solution of arsenic at a dose of 2 $mg(As).dm^{-3}$ resulted in significant signs of toxicity.

In contrast to the mentioned observations, no wilting or chlorosis of leaves was observed in our experiment at the given doses of As and Cd. The stressed plants of the cv. Cordoba showed faster growth. However, this was not reflected in the final shoot biomass (neither fresh weight nor dry matter) (Table 1). On the other hand, we have scored a significant reduction in dry weight of shoots of the cv. Bólyi 44 (by 24, 34%) exposed to Cd, but at the same time no differences in lengths and fresh weights of stressed- versus control shoots. Thus, the impact of the two metals on the growth of tested soybean plants was relatively mild, possibly given by bioavailability of metals in the soil used.

The exposure to metals, however, resulted in significantly elevated amounts of both As and Cd accumulated in the soybean shoots (Table 2). Clearly, the plants of the cv. Bólyi 44 took up significantly higher amounts of metal comparing to the cv. Cordoba ($p<10^{-5}$). Nevertheless, based on a survey on Cd-accumulation capacity of 150 Japanese soybean cultivars (Sugiyama *et al*., 2011) both the cv. Bólyi 44 and the cv. Cordoba represent a low-accumulating cultivar type. In the mentioned work the average concentrations of Cd in shoots for the low- and high accumulating soybean groups were 2.19 (± 0,02) $\mu g.g^{-1}$ and 4.16 (± 0,11) $\mu g.g^{-1}$, respectively. Accumulation of metals in entire shoots (and later on seeds) is determined by the ability to accumulate the metal in roots and is given genetically (Sugiyama *et al*., 2007).

**Table 1. Effect of arsenic (As) and cadmium (Cd) on the selected physiological parameters of shoots of the cvs. Bólyi 44 and Cordoba with respect to corresponding controls (C).**

| Cultivar | Treatment | length | FW | DW |
|---|---|---|---|---|
| Bólyi 44 | As | 21,52 ± 1,93 $^{n.s.}$ | 1,17 ± 0,22 $^{n.s.}$ | 0,15 ± 0,06 $^{A}$ |
| | Cd | 21,27 ± 2,23 $^{n.s.}$ | 1,23 ± 0,29 $^{n.s.}$ | 0,12 ± 0,04 $^{B}$ |
| | C | 20,93 ± 1,77 $^{n.s.}$ | 1,26 ± 0,22 $^{n.s.}$ | 0,15 ± 0,04 $^{A}$ |
| Cordoba | As | 31,03 ± 5,20 $^{A}$ | 1,60 ± 0,40 $^{n.s.}$ | 0,21 ± 0,07 $^{n.s.}$ |
| | Cd | 30,34 ± 3,79 $^{A}$ | 1,57 ± 0,30 $^{n.s.}$ | 0,22 ± 0,06 $^{n.s.}$. |
| | C | 28,10 ± 2,54 $^{B}$ | 1,64 ± 0,27 $^{n.s.}$ | 0,22 ± 0,07 $^{n.s.}$ |

Values followed by different letters in the same column for a genotype indicate a significant difference at $p<0.05$; n.s.: no significant difference between treatments

**Table 2. Content of As and Cd (in µg.g$^{-1}$) in the shoots of the studied plants.**

| Metal (oid) | Bólyi 44 | | | Cordoba | | |
|---|---|---|---|---|---|---|
| | control | metal-stressed | fold increase | control | metal stressed | fold increase |
| Arsenic | 0,31 ± 0,00 | 1,18 ± 0,22 *** | 3,8 | 0,31 ± 0,00 | 0,87 ± 0,19 ** | 2,8 |
| Cadmium | 0,12 ± 0,03 | 5,82 ± 0,73 *** | 46,9 | 0,21 ± 0,02 | 2,72 ± 0,35 *** | 13,3 |

Asterisks indicate significant difference with respect to control values at $p<0.01$ (**) or $p<0.001$ (***)

In the leaves of both the soybean cultivars we investigated the effects of heavy metals on the leaf pigment contents, since it is considered as a marker of physiological state of plants during not only development and senescence, but also acclimation and adaptation to different environments and stresses including heavy metals (Young & Britton, 1990). According to several authors (Li *et al*., 2009) changes in the concentration of Chl*a* and *b* and particularly changes in their ratio are an equal important parameter for estimating the effect of an environmental parameter in plants. However, in the leaves (both unifoliar and trifoliar) of the Cd-treated plants of the cv. Cordoba we found that the content of Chl*a*, the sum of (Chl*a* + Chl*b*) and the Chl*a/b* weight ratio (unifoliar leaves only) was negatively affected (Table 3), as observed also by others (Ouzounidou *et al*., 1997; Raziuddin *et al*., 2011). In contrast, the contents of Chl*b* or carotenoids remained unaltered. Previously it has been shown that ions of cadmium cause chlorophyll *a* to degrade more rapidly than chlorophyll *b* (Oliveira *et al*., 1994; Abdel-Basset *et al*., 1995; Kummerová *et al*., 2010). The reduction in chlorophylls (Kang *et al*., 2007; Shah *et al*., 2008) under cadmium stress has been attributed to disruption of the chloroplast structure (Barceló *et al*., 1988), direct inhibition of the activity of sulfhydryl requiring enzymes such as ALA (δ-aminolevulinate)-synthase and ALA-dehydratase (important for tetrapyrrole biosynthetic pathway leading to chlorophyll formation) and RUBISCO (Mobin & Khan, 2007). Since the Chl*a/b* weight ratio is an indicator of the functional pigment equipment and light adaptation/acclimation of the photosynthetic apparatus, monitoring of its decreasing can be used as an early warning system for the toxic effect of metal accumulation in plants (Li *et al*., 2009). Nevertheless, altered content of a single photosystem component does not appear to be a sufficient indicator for occurring intoxication.

Our data (Tables 1 and 3) are in agreement with previous observations that Car content is affected to lesser extent than Chl content (Stoeva *et al*., 2005). In contrast they do not seem to support the hypothesis that in young metal-stressed plants the photosynthesis is inhibited to less extent than growth (Merakchiyska & Yordanov 1983; Stoeva *et al*., 2005).

Inspite that except for the few above mentioned metal-related changes no other ones were detected in neither of the 2 cultivars (Table 3). The carotenoid and other pigment levels in the leaves of the cv. Bólyi 44 were significantly higher when compared with the cv. Cordoba (Table 4). This difference between the plants of the 2 cultivars was significant in case of both control- as well as heavy metal-stressed plants. This fact might have serious consequences for capability to fate stress conditions since the photosynthetic equipment directly determines the energetically status of plants as a prerequisite for activate defense (Drazkiewicz *et al*., 2003; Stamp, 2003). In addition, under normal conditions carotenoid retention in the progress of Chl breakdown directly contributes to photoprotection during leaf senescence (Merzlyak & Gitelson, 1995). The higher carotenoid biosynthesis/content of leaves of the cv. Bólyi 44 is a genetic characteristic and might indicate to relatively better defense equipment against stresses (Middleton & Teramura, 1993). Thus, these results in our study support the previously described genotype-dependent response of plants to heavy metals (Metwally *et al*., 2005; Sugiyama *et al*., 2011).

**Table 3. Contents of photosynthetic pigments in the leaves of two soybean cultivars exposed to heavy metals.**

| Leaf type | Pigment content (mg.g$^{-1}$ of fresh weight) | | | | | |
|---|---|---|---|---|---|---|
| | cv. Bólyi 44 | | | cv. Cordoba | | |
| | control | As 5 | Cd 50 | control | As 5 | Cd 50 |
| | **Chlorophyll *a*** | | | | | |
| Unifoliar | 1,83 ± 0,54 $^{A}$ | 1,66 ± 0,10 $^{A}$ | 1,75 ± 0,40 $^{A}$ | 1,47 ± 0,32 $^{A}$ | 1,46 ± 0,18 $^{A}$ | 1,16 ± 0,29 $^{B}$ |
| Trifoliar | 1,70 ± 0,45 $^{A}$ | 1,45 ± 0,11 $^{A}$ | 1,62 ± 0,44 $^{A}$ | 1,54 ± 0,25 $^{A}$ | 1,48 ± 0,23 $^{AB}$ | 1,25 ± 0,15 $^{B}$ |
| | **Chlorophyll *b*** | | | | | |
| Unifoliar | 0,58 ± 0,19 $^{A}$ | 0,44 ± 0,13 $^{A}$ | 0,50 ± 0,17 $^{A}$ | 0,48 ± 0,11 $^{A}$ | 0,46 ± 0,04 $^{A}$ | 0,45 ± 0,10 $^{A}$ |
| Trifoliar | 0,45 ± 0,13 $^{A}$ | 0,39 ± 0,11 $^{A}$ | 0,46 ± 0,10 $^{A}$ | 0,43 ± 0,10 $^{A}$ | 0,45 ± 0,07 $^{A}$ | 0,38 ± 0,06 $^{A}$ |
| | **Carotenoids** | | | | | |
| Unifoliar | 0,54 ± 0,19 $^{A}$ | 0,46 ± 0,08 $^{A}$ | 0,49 ± 0,18 $^{A}$ | 0,37 ± 0,11 $^{A}$ | 0,37 ± 0,08 $^{A}$ | 0,36 ± 0,11 $^{A}$ |
| Trifoliar | 0,45 ± 0,09 $^{A}$ | 0,41 ± 0,07 $^{A}$ | 0,49 ± 0,22 $^{A}$ | 0,43 ± 0,06 $^{A}$ | 0,35 ± 0,12 $^{A}$ | 0,36 ± 0,10 $^{A}$ |
| | **Chlorophyll (*a*+*b*)** | | | | | |
| Unifoliar | 1,14 ± 0,30 $^{A}$ | 1,06 ± 0,07 $^{A}$ | 1,11 ± 0,22 $^{A}$ | 0,97 ± 0,17 $^{A}$ | 0,96 ± 0,09 $^{A}$ | 0,79 ± 0,14 $^{B}$ |
| Trifoliar | 1,07 ± 0,25 $^{A}$ | 0,90 ± 0,08 $^{A}$ | 0,99 ± 0,28 $^{A}$ | 0,99 ± 0,16 $^{A}$ | 0,96 ± 0,13 $^{A}$ | 0,82 ± 0,09 $^{B}$ |
| | **Chlorophyll *a*/*b*** | | | | | |
| Unifoliar | 3,72 ± 1,07 $^{A}$ | 3,60 ± 0,73 $^{A}$ | 4,34 ± 0,91 $^{A}$ | 3,46 ± 0,62 $^{A}$ | 3,03 ± 0,36$^{B}$ | 2,78 ± 0,45 $^{B}$ |
| Trifoliar | 4,01 ± 0,93 $^{A}$ | 4,14 ± 0,97 $^{A}$ | 3,86 ± 0,78 $^{A}$ | 3,44 ± 0,55 $^{A}$ | 3,31 ± 0,58 $^{A}$ | 3,28 ± 0,55 $^{A}$ |
| | **Chlorophyll (*a*+*b*)/ Carotenoids** | | | | | |
| Unifoliar | 2,14 ± 0,40 $^{A}$ | 2,31 ± 0,37$^{A}$ | 2,14 ± 0,25 $^{A}$ | 2,51 ± 0,42 $^{A}$ | 2,65 ± 0,38 $^{A}$ | 2,52 ± 0,23 $^{A}$ |
| Trifoliar | 2,25 ± 0,40 $^{A}$ | 2,23 ± 0,28 $^{A}$ | 2,25 ± 0,30 $^{A}$ | 2,31 ± 0,32 $^{A}$ | 2,53 ± 0,27 $^{A}$ | 2,55 ± 0,41 $^{A}$ |

Values followed by different letters in the same column for a genotype indicate a significant difference at $p<0.05$

**Table 4. Overview of the results of the 2-way ANOVA analysis on the pigment content data from metal-exposed soybean leaves.**

| | Chl a | | | Chl b | | | Car | | | Chl(a+b) | | | Chla/b | | | Chl(a+b)/car | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *G* | *LT* | *I* | *G* | *LT* | *I* | *G* | *LT* | *I* | *G* | *LT* | *I* | *G* | *LT* | *I* | *G* | *LT* | *I* |
| As | * | * | * | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | * | * | n.s. | * | n.s. | n.s. | * | n.s. | n.s. |
| Cd | * | n.s. | n.s. | n.s. | n.s. | n.s. | * | n.s. | n.s. | * | n.s. | n.s. | * | n.s. | n.s. | n.s. | n.s. | n.s. |

Asterisks indicate significant effect of the genotype (*G*), leaf type (*LT* - unifoliar or trifoliar) or their interaction (*I*) at $p<0.01$ (**) or $p<0.5$ (*). No significant effect is indicated as n.s.

The data obtained in this study further pointed differences in responses when the 2 leaf types (unifoliar and trifoliar) were compared, or when the interaction of leaf type and genotype was assayed. For example, the decrease of the Chl*a* content upon. As treatment was not significant with respect to corresponding controls in case of the cv. Bólyi 44 (Table 3). However, it appeared to be significantly different between the unifoliate and trifoliate leaf of the same plant (Table 4). In this case the interaction of genotype and leaf type was significant as well (Table 4). Previously the close relationship between the age and type of the leaf tissue and response of the photosynthetic apparatus under Cd stress has been shown (Drazkiewicz *et al*., 2003; Barceló *et al*., 1988). The phenomenon has partially been attributed to differences in accumulation of glutathione and phytochelatins involved in metal chelatation and detoxification (Drazkiewicz *et al*., 2003). A trade-off between development (growth) and defence has been presumed and a range of theories exist that try to explain spatial and temporal variation in plant defense (reviewed in Stamp, 2003).

## Conclusions

Cadmium accumulated in shoots of soybean increased the length of shoots of the cv. Cordoba, while arsenic caused significant decrease of dry weight of shoots of the cv. Bólyi 44. Nevertheless, the signs of occurring stress were mild and out of the photosynthetic pigments analysed only the content of chlorophyll *a* was negatively affected in the leaves of the cv. Cordoba. The genotype dependent response to heavy metals can be attributed to several factors (e.g. to the content of phytochelatins involved in metal detoxification), while in this work it coincides with the content of photosynthetic pigments e.g. carotenoids that are effective antioxidative

protectants. The results of our analyses suggest that pigment content can be a more sensitive and perhaps reliable indicator for plant damage by heavy metals than plant growth parameters since the negative effect cannot be readily observed. Further experiments on a larger genotype set would be required to identify the possible correlation between carotenoids and metal tolerance. Furthermore, plant response apparently varies in different plant parts. Therefore, specificities of plant defense mechanisms are important to consider to efficiently avoid/eliminate heavy metal pollution.

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**References**

Abdel-Basset, R., A.A. Issa and M.S. Adam. 1995. Chlorophyllase activity: effect of heavy metals and calcium. *Photosynthetica*, 31: 421-425.

Abedin, M.J. and A.A. Meharg. 2002. Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.). *Plant Soil*, 243: 57-66.

Ahmad, I., M.J. Akhtar, Z.A. Zahir and A. Jamil. 2012. Effect of cadmium on seed germination and seedling growth of four wheat (*Triticum aestivum* L.) cultivars. *Pak. J. Bot*., 44: 1569-1574.

Bačkor, M., J. Kováčik, J. Piovar, T. Pisani and S. Loppi. 2010. Physiological aspects of cadmium and nickel toxicity in the lichens *Peltigera rufescens* and *Cladina arbuscula* subsp. *mitis*. *Water Air Soil Poll.*, 207: 253-262.

Barceló, J., M.D. Vázquez and C. Poschenrieder. 1988. Cadmium-induced structural and ultrastructural-changes in the vascular system of bush bean stems. *Bot. Acta*, 101: 254-261.

Bavi, K., B. Kholdebarin, A. Moradshahi. 2011. Effect of cadmium on growth, protein content and peroxidase activity in pea plants. *Pak. J. Bot.*, 43: 1467-1470.

Carbonell-Barrachina, A.A., F. Burló and J. Mataix. 1998. Response of bean micronutrient nutrition to arsenic and salinity. *J. Plant Nutr*., 21: 1287-1299.

Curran, P.J., J.L. Dungan and H.L. Gholz. 1990. Exploring the relationship between reflectance red edge and chlorophyll content in slash pine. *Tree Physiol.*, 7: 33-48.

Dragun, A.K. 1998. The agricultural production of environment – Some Nordic issues. *Swed. J. Agr. Res.*, 28: 49-55.

Drazkiewicz, M., A. Tukendorf and T. Baszynski. 2003. Age-dependent response of maize leaf segments to cadmium treatment: Effect on chlorophyll fluorescence and phytochelatin accumulation. *J. Plant Physiol.*, 160: 247-254.

Filella, I., L. Serrano, J. Serra and J. Penuelas. 1995. Evaluating wheat nitrogen status with canopy reflectance indexes and discriminant-analysis. *Crop Sci.*, 35: 1400-1405.

Hendry, G.A.F., J.D. Houghton and S.B. Brown. 1987. Tansley review no-11 – the degradation of chlorophyll – a biological enigma. *New Phytol.*, 107: 255-302.

Hronec, O. 1996. *Exhaláty-pôda-vegetácia*. Monograph, TOP s.r.o., Prešov (Slovakia).

Kang, W., I.H. Shamsi and G.P. Zhang. 2007. Synergistic interaction of NaCl and Cd on growth and photosynthetic parameters in soybean genotypes differing in salinity tolerance. *J. Zhejiang Univ.-Sc. B*, 8(4): 266-271.

Kazantzis, G. 1987. Cadmium. In: *Genotoxic and carcinogenic metals: Environmental and Occupational Occurence and Exposure*. (Eds.): L. Fishbein, A. Furst, M.A. Mehlman. Advances in modern Environmental Toxicology, 11, Princeton Scientific Publishing Co, Princeton, N. J., pp. 127-143.

Kummerová, M., Š. Zezulka, K. Kráľová and E. Masarovičová. 2010. Effect of zinc and cadmium on physiological and production characteristics in *Matricaria recutita*. *Biol. Plantarum*, 54(2): 308-314.

Li, C., S.I. Feng, Y. Shao, L.N. Jiang, X.Y. Lu and X.I. Hou. 2007. Effects of arsenic on seed germination and physiological activities on wheat seedlings. *J. Environ. Sci.-China*, 19: 725-732.

Li, Y., J. Liu, Y. Liu and X. Li. 2009. Effects of EDTA on mechanism of lead accumulation in *Typha orientalis* Presl. *B. Environ. Contam. Tox.*, 83: 553-557.

Lichtenthaler, H.K. and A.L. Wellburn. 1985. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. T.*, 11: 591-592.

Liu, X.L., S.Z. Zhang, X.Q. Shan and Y.G. Zhu. 2005. Toxicity of arsenate and arsenite on germination, seedling growth and amylolytic activity of wheat. *Chemosphere*, 61: 293-301.

Merakchiyska, M. and I. Yordanov. 1983. Influence of some heavy metals on the growth, content of plastid pigments, and the photosynthetic activity in bean plants. *Scientific Conference on Botany*, BASC, Sofia (Bulgary), pp. 848-851.

Merzlyak, M.N. and A. Gitelson. 1995. Why and what for the leaves are yellow in autumn- on the interpretation of optical –spectra of senescing leaves (*Acer platanoides* L.). *J. Plant Physiol.*, 145: 315-320.

Merzlyak, M.N., A.A. Gitelson, O.B. Chivkunova and V.Y. Rakitin. 1999. Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening. *Physiol. Plantarum*, 106: 135-141.

Mészáros, P., L. Rybanský, P. Hauptvogel, R. Kuna, J. Libantová, J. Moravčíková, B. Piršelová, A. Tirpáková, I. Matušíková. 2012. Cultivar-specific kinetics of chitinase induction in soybean roots during exposure to arsenic. *Mol. Biol. Rep.*, doi 10.1007/s11033-012-2271-y.

Metwally, A., V.I. Safronova, A.A. Belimov and K.J. Dietz. 2005. Genotypic variation of the response to cadmium toxicity in *Pisum sativum*. *J. Exp. Bot.*, 56: 167-178.

Middleton, E.M. and A.H. Teramura. 1993. The role of flavonol glycosides and carotenoids in protecting soybean from ultraviolet-B damage. *Plant Physiol.*, 103: 741-752.

Mobin, M. and N.A. Khan. 2007. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. *J. Plant Physiol.*, 164: 601-610.

Moran, J.A., A.K. Mitchell, G. Goodmanson and K.A. Stockburger. 2000. Differentiation among effects of nitrogen fertilization treatments on conifer seedlings by foliar reflectance: a comparison of methods. *Tree Physiol.*, 20: 1113-1120.

Oliveira, J.A., M.A. Oliva and J. Cambraia. 1994. Effects of cadmium on chlorophyll contents and on peroxidase activity in soybean. *Rev. Bras. Fisiol. Vegetal*, 6(2): 97-101.

Ouzounidou, G., M. Moustakas and E.P. Eleftheriou. 1997. Physiological and ultrastructural effects of cadmium on wheat (*Triticum aestivum* L.) leaves. *Arch. Environ. Con. Tox.*, 32: 154-160.

Ozdener, Y. and H.G. Kutbay. 2011. Physiological and biochemical responses of the leaves of *Verbascum wiedemannianum* Fisch. & Mey. to cadmium. *Pak. J. Bot*., 43: 1521-1525.

Peralta, J.R., J.L. Gardea-Torresdey, K.J. Tiemann, E. Gomez, S. Arteaga, E. Rascon and J.G. Parsons. 2001. Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). *B. Env. Con. Tox.*, 66: 727-734.

Pisani, T., S. Munzi, L. Paoli, M. Bačkor, J. Kováčik and S. Loppi. 2011. Physiological effects of mercury in the lichens *Cladonia arbuscula* subsp. *mitis* (Sandst.) Ruoss and *Peltigera rufescens* (Weiss) Humb. *Chemosphere*, 82: 1030-1037.

Rahman, M.A., H. Hasegawa, M.M. Rahman, M.A. Rahman and M.A.M. Miah. 2007. Accumulation of arsenic in tissues of rice plant (*Oryza sativa* L.) and its distribution in fractions of rice grain. *Chemosphere*, 69: 942-948.

Raziuddin, Farhatullah, G. Hassan, M. Akmal, S.S. Shah, F. Mohammad, M. Shafi, J. Bakht and W. Zhou. 2011. Effects of cadmium and salinity on growth and photosynthesis parameters of *Brassica* species. *Pak. J. Bot*., 43: 333-340.

Rühling, A. 1994. *Atmospheric heavy metal deposition in Europe estimations based on moss analyses*. NORD, pp. 9.

Shafi, M., G.P. Zhang, J. Bakht, M.A. Khan, Ejaz-ul-Islam, M.D. Khan and Raziuddin. 2010. Effect of cadmium and salinity stresses on root morphology of wheat. *Pak. J. Bot*., 42: 2747-2754.

Shah, F.R., N. Ahmad, K.R. Masood and D.M. Zahid. 2008. The influence of cadmium and chromium on the biomass production of shisham (*Dalbergia sissoo* Roxb.) seedlings. *Pak. J. Bot*., 40: 1341-1348.

Shri, M., S. Kumar, D. Chakrabarty, P.K. Trivedi, S. Mallick, P. Misra, D. Shukla, S. Mishra, S. Srivastava, R.D. Tripathi and R. Tuli. 2009. Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedlings. *Ecotox. Environ. Safe.*, 72: 1102-1110.

Stamp, N. 2003. Out of the quamire of plant defense hypotheses. *Q. Rev. Biol.*, 78: 23-55.

Stoeva, N., M. Berova and Z. Zlatev. 2005. Effect of arsenic on some physiological parameters in bean plants. *Biol. Plantarum*, 49: 293-296.

Sugiyama, M., N. Ae and M. Hajika. 2011. Developing of a simple method for screening soybean seedling cadmium accumulation to select soybean genotypes with low seed cadmium. *Plant Soil*, 341: 413-422.

Sugiyama, M., N. Ae and T. Arao. 2007. Role of roots in differences in seed cadmium concentration among soybean cultivars-proof by grafting experiment. *Plant Soil*, 295: 1-11.

Verbruggen, N., C. Hermans and H. Schat. 2009. Mechanisms to cope with arsenic or cadmium excess in plants. *Curr. Opin. Plant Biol.*, 12: 364-372.

Young, A.J. and G. Britton. 1990. Carotenoids and oxidative stress. In: *Current Research in Photosynthesis*. (Ed.): M. Baltscheffsky. Kluwer, Dordrecht, pp. 587-590.

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