

## ***Cannabis sativa* L. growing on heavy metal contaminated soil: growth, cadmium uptake and photosynthesis**

P. LINGER<sup>\*1</sup>, A. OSTWALD\* and J. HAENSLER\*\*

*Physiological Chemistry of Plants, Dept. C - Mathematics and Science, Bergische University of Wuppertal, Gauss Str. 20, D-42097 Wuppertal, Germany\**  
*Geobotany, Heinrich-Heine-University, Universitätsstr. 1, D-40225 Düsseldorf\*\**

### **Abstract**

The effects of different cadmium concentrations [17 mg(Cd) kg<sup>-1</sup>(soil) and 72 mg(Cd) kg<sup>-1</sup>(soil)] on *Cannabis sativa* L. growth and photosynthesis were examined. Hemp roots showed a high tolerance to Cd, *i.e.* more than 800 mg(Cd) kg<sup>-1</sup>(d.m.) in roots had no major effect on hemp growth, whereas in leaves and stems concentrations of 50 - 100 mg(Cd) kg<sup>-1</sup>(d.m.) had a strong effect on plant viability and vitality. For control of heavy metal uptake and xylem loading in hemp roots, the soil pH plays a central role. Photosynthetic performance and regulation of light energy consumption were analysed using chlorophyll fluorescence analysis. Seasonal changes in photosynthetic performance were visible in control plants and plants growing on soil with 17 mg(Cd) kg<sup>-1</sup>(soil). Energy distribution in photosystem 2 is regulated in low and high energy phases that allow optimal use of light and protect photosystem 2 from over-excitation, respectively. Photosynthesis and energy dissipation were negatively influenced by 72 mg(Cd) kg<sup>-1</sup>(soil). Cd had detrimental effects on chlorophyll synthesis, water splitting apparatus, reaction centre, antenna and energy distribution of PS 2. Under moderate cadmium concentrations, *i.e.* 17 mg(Cd) kg<sup>-1</sup>(soil), hemp could preserve growth as well as the photosynthesis apparatus, and long-term acclimation to chronically Cd stress occurred.

*Additional key words:* acclimation, chlorophyll fluorescence, phytoextraction, quenching, tolerance.

### **Introduction**

In the future, one key problem for foodstuff production and growth of renewable resources will be the climatic changes in areas of intensive agriculture. These changes will lead to the necessity of cultivating crops on polluted ground, because of decreasing amounts of agricultural arable land generated by the expansion of arid areas. Heavy metals are one of the key factors that exert negative influences on man and the environment. Moreover, heavy metal release into the environment is, for the most part, a man-made problem and for cadmium (Cd) alone this has been estimated to be 29 190 t year<sup>-1</sup> worldwide (Sanita di Toppi and Gabbrielli 1999). This pollution is caused by smelting, sewage sludge distribution and automobile emissions (Foy *et al.* 1978,

Adriano 1986, Chronopoulos *et al.* 1997, Saxena *et al.* 1999, Dahmani-Muller *et al.* 2000); however, naturally occurring heavy metal enriched soil has been found all over the world (Allaway 1968, Adriano 1986).

The devastating effects of heavy metals on plants have been described by numerous authors (Ouzounidou *et al.* 1997, Jiang and Liu 2000, Baryla *et al.* 2001, Seregin and Ivanov 2001, Kevrešan *et al.* 2003, Khudsar *et al.* 2004, Mazen 2004, Šimonovičová *et al.* 2004). Of these, Cd is known to be one of the most phytotoxic heavy metals (Prasad 1995, Salt *et al.* 1995a). Heavy metal toxic effects in plants depend on the physico-chemical properties of the soil, which determine metal availability (Ernst 1996), as well as the genetic capacity

Received 14 November 2003, accepted 24 January 2005.

*Abbreviations:* AAS - atomic absorption spectroscopy; Cd1, Cd2 - cadmium concentration 1, 2; d.m. - dry mass; ET - electron transport; f.m. - fresh mass; PPF - photosynthetic photon flux density; PS 2 - photosystem 2; ΔpH - proton gradient; Φ<sub>PS2</sub> - quantum efficiency of photosystem 2; q<sub>P</sub> - photochemical quenching; q<sub>N</sub> - non-photochemical quenching, q<sub>E</sub> - energy dependent quenching; q<sub>T</sub> - quenching related to state transition; q<sub>I</sub> - photoinhibitory quenching, q<sub>F</sub> - fast-relaxing non-photochemical quenching.

*Acknowledgement:* We thank Mr. Steffi-Wellie from the Deutsche Saatgut Veredlung for providing us with *Cannabis sativa* L. USO31 seeds.

<sup>1</sup> Corresponding author; fax: (+49) 202 439 3142, e-mail: linger@pinea.de

of the plant to react to abiotic stressors (Sanita di Toppi and Gabbriellini 1999). Resistant plants are able to tolerate heavy metals or to avoid their uptake (Ernst 1976, Greger 1999); whereas in heavy metal sensitive plants, membranes and many biochemical processes, like respiration and photosynthesis, are affected (for reviews see Rama Devi and Prasad 1999, Seregin and Ivanov 2001). Cd influences photosynthesis in two ways: 1) indirectly through disturbance of water and ion uptake which consequently negatively affects the plant water status (Seregin and Ivanov 2001), and 2) by directly affecting the chloroplast apparatus after entering the leaf cells. In the latter case, the heavy metal interferes with chlorophyll synthesis, assembly of pigment protein complexes and thylakoids, the electron transport chain (ET chain), Calvin cycle enzymes, sugar transport and consumption, chloroplast replication and oxidative stress (Böddi *et al.* 1995, Horvath *et al.* 1996, Dubey 1997, Dietz *et al.* 1999, Barylka *et al.* 2001, Seregin and Ivanov 2001).

The most effective way to analyse the influence of stressors on photosynthesis *in vivo* is to detect chlorophyll fluorescence and to evaluate the quenching components as these methods have the advantage of being both non-invasive and non-destructive (Schreiber and Bilger 1987). Here, the parameters  $F_0$  (initial fluorescence in the dark when all reaction centres are open),  $F_m$  (maximum fluorescence in dark-adapted state) and the resulting  $F_v/F_m = (F_m - F_0)/F_m$  were estimated; all of them can be used as an initial indication of plant stress

## Materials and methods

*Cannabis sativa* L. cv. USO31, a fibre hemp cultivar, was cultivated in commercial available soil in pots (CompoSANA®, pH = 5.5 - 6.5) in a greenhouse in Wuppertal (Germany). The soil was partly artificially contaminated with Cd in the form of CdSO<sub>4</sub> to two different concentrations. Before beginning the experiment, we took six samples from each pot ground and evaluated the Cd concentration available to the plant: Cd concentration 1 (Cd1) 17.3 ± 2.0 mg(Cd) kg<sup>-1</sup>(soil) and Cd concentration 2 (Cd2) 71.7 ± 8.2 mg(Cd) kg<sup>-1</sup>(soil). Moreover, we also determined the soil pH using a soil water extract. Briefly, 10 g soil were passed through a 2 mm sieve and then suspended in 25 cm<sup>3</sup> H<sub>2</sub>O (bidistilled) for 2 h before the pH values were measured: Cd1 pH = 4.40 ± 0.05 and Cd2 pH = 5.55 ± 0.07, control soil pH = 5.5 - 6.5.

Plants were fertilised weekly with equal amounts of a commercial N-P-K fertiliser (7 - 5 - 6 %). The growth conditions were 23 - 25 °C and 50 - 70 % relative humidity. The photoperiod was 16 h under natural light supplemented with artificial irradiation of a minimal photosynthetic photon flux density (PPFD) of

(Björkman and Demmig 1987, Bolhar-Nordenkamp *et al.* 1989). To gain a more detailed insight into the physiological action of stressors, the resolution of chlorophyll quenching into the quenching components was also assessed. The quantum efficiency of photosystem 2 (PS 2),  $\Phi_{PS2}$ , (Genty *et al.* 1989), and photochemical quenching,  $q_p$ , reflecting the number of open reaction centres, are indicators for the capacity of photochemical processes. Non-photochemical quenching component,  $q_N$ , unites processes that are associated with heat dissipation and most of the time reversible inactivation of PS 2 reaction centres to prevent destruction of the photosynthesis apparatus (Krause and Weis 1991, Horton *et al.* 1996).  $q_N$  consists of the parameters  $q_E$  (energy dependent quenching),  $q_T$  (quenching related to state transition) and  $q_I$  (photoinhibitory quenching).  $q_E$  and  $q_T$  can be combined to  $q_F$ , fast-relaxing non-photochemical quenching (Linger and Brüggemann 1999).  $q_T$  is only of minor importance for  $q_N$  formation (Ruban and Horton 1995), and  $q_E$  is the main component of non-photochemical quenching (Krause and Weis 1991, Horton *et al.* 1996).

To assess the effects of Cd to a renewable resource, we chose *Cannabis sativa* as a model plant. Here, we answer the following questions: a) Is hemp a Cd tolerant plant? b) Does Cd interfere in the photosynthetic apparatus and the regulation of photosynthetic energy dissipation?

100  $\mu\text{mol m}^{-2}\text{s}^{-1}$  during the day. For each concentration, 90 seeds were sown and divided into 3 independent sets. The germination rates were determined 10 d after sowing day by counting seedlings. Control plants were cultivated under the same conditions in soil without Cd.

Whole plants were harvested 10 d after the seeds were sown and weighed immediately to avoid transpiration loss for fresh mass (f.m.) determination. Subsequently, we collected plants each week for 7 weeks. The last matured plants were harvested 133 d after the beginning of the trial. Each time 3 - 6 plants were collected (exceptions are indicated in the text). After fresh mass determination, the plant material was frozen and either stored at -20 °C until drying or dried immediately at 60 °C.

To examine soil Cd concentrations, soil samples were passed through a 2 mm sieve and 5 g were extracted in 50 cm<sup>3</sup> 1 M ammonium-acetate for 2 h, after which samples were diluted with 3.9 % HNO<sub>3</sub> and then subjected to atomic absorption spectroscopy (AAS, Analyst 100, Perkin-Elmer, Rodgau - Jügesheim, Germany).

To determine Cd concentrations in plant material, the

material was dried and powdered with a mill and then redried at 60 °C for 48 h. Approximately 100 mg of the homogenised plant material were transferred to a *Teflon* reaction vessel, to which 3 cm<sup>3</sup> of 65 % HNO<sub>3</sub> were added, before being incubated at 50 °C for 2.5 h, and then for 7 h at 180 °C. After cooling, the residual solution was diluted with H<sub>2</sub>O (bidistilled) to a final volume of 50 cm<sup>3</sup> and Cd was determined by AAS.

Chlorophyll fluorescence of leaf discs, taken from the first fully expanded leaf (from the top), were detected by a *PAM 101 fluorometer* (Walz, Effeltrich, Germany). Six to 8-week-old plants were used for light response experiments and leaves from days 13 to 97 after sowing were used to assess Cd effects on photosynthesis. Measurement conditions were constant at 20 °C in water vapour saturation with 21 % O<sub>2</sub> and atmospheric CO<sub>2</sub>. Leaf discs were allowed to adapt to the dark for 15 min prior to F<sub>0</sub>, F<sub>m</sub> determination (Schreiber *et al.* 1986), followed by a 15 min actinic “white light” of 500 μmol m<sup>-2</sup>s<sup>-1</sup> PPFD (the experiments with Cd-treated plants). PPFD of 56 to 3 000 μmol m<sup>-2</sup>s<sup>-1</sup> were used for light response experiments. At the end of the illumination phase, the leaf discs were exposed to a saturating flash (10 000 μmol m<sup>-2</sup>s<sup>-1</sup>). To monitor relaxation of non-

photochemical quenching, plant material then underwent a 15 min dark period followed by repetitive saturating flashes every 100 s. Chlorophyll fluorescence quenching parameters were calculated as according to Schreiber *et al.* (1986), Walters and Horton (1991) for q<sub>p</sub>, q<sub>N</sub>, q<sub>I</sub> and Linger and Brüggemann (1999) for q<sub>F</sub>, defined as q<sub>F</sub> = (q<sub>N</sub> - q<sub>I</sub>)/(1 - q<sub>I</sub>), and Φ<sub>PS2</sub> as according to Genty *et al.* (1989) Φ<sub>PS2</sub> = F<sub>v</sub>'/F<sub>m</sub>' × q<sub>p</sub>. F<sub>v</sub>'/F<sub>m</sub>' determines the quantum efficiency of open reaction centres, and q<sub>p</sub>, photochemical quenching, reflects the number of open reaction centres.

After the chlorophyll fluorescence measurements, the leaf discs were prepared for chlorophyll content determination in 80 % acetone and analysed according to Arnon (1949).

All data shown represent means and standard deviation of 3 - 6 plants; exceptions for Cd2 plant data are indicated in the text. In this case, after day 20, only one Cd2 plant could be used for measurements because of total necrotic leaves of the other plants and this should be borne in mind when interpreting these results. However, Cd2 plant data still provided an indication of the plant's response when lethal Cd concentrations were applied. The *t*-tests were performed using *SPSS SigmaPlot 8.0*.

## Results

The germination rate was 70 % for the control plants compared to 79 and 77 % for the Cd1 and the Cd2 plants, respectively. Cd concentrations of up to 72 mg kg<sup>-1</sup>(soil) had no negative effect on germination. A significant difference was observed for germination rates between the Cd1 and the control plants (*P* < 0.05); however, no significant difference was observed for germination between Cd1 and Cd2 grown plants nor the control and Cd2 plants.

The Cd contamination led to a significant growth inhibition, demonstrated through the almost double as high biomass after 59 d of the control plants

compared to the Cd1 plants (Fig. 1). However, at the end of the vegetation period, the control and the Cd1 plants had the same fresh mass (Fig. 1, control: 115 - 220 g, Cd1: 73 - 310 g), with great fluctuations being observed in both populations as indicated by the dry mass (d.m.) values (control: 33 - 58 g, mean 50 g; Cd1: 11 - 83 g, mean 51 g).

The Cd2 plants displayed a very strong growth inhibition, and most plants died 4 to 5 weeks after sowing. Furthermore, the plants collected after 38 d were almost necrotic, and the fresh mass could practically be assigned to the stems only. Indeed, only one plant survived this Cd treatment until 80 d. The biomass production was negligible.

The roots always accumulated the highest Cd concentrations (Fig. 2), which reached a maximum of 830 mg(Cd) kg<sup>-1</sup>(d.m.) in Cd1 plants after 24 d, and then began to decline with plant growth. Stems and leaves accumulated Cd to a much lesser extent; the highest determined values were 87 and 68 mg(Cd) kg<sup>-1</sup>(d.m.) in stem and leaves, respectively. At the end of the vegetation period, the means were 42 mg(Cd) kg<sup>-1</sup>(d.m.) for roots, 20 mg(Cd) kg<sup>-1</sup>(d.m.) for stems and 15 mg(Cd) kg<sup>-1</sup>(d.m.) for leaves. With the exception of the roots, Cd2 plants always accumulated higher Cd quantities in the other plant parts (Fig. 2).

In the stems, heavy metal concentrations were approximately 4 - 5 times higher in the Cd2 plants compared to those of Cd1 (Fig. 2). In addition, a

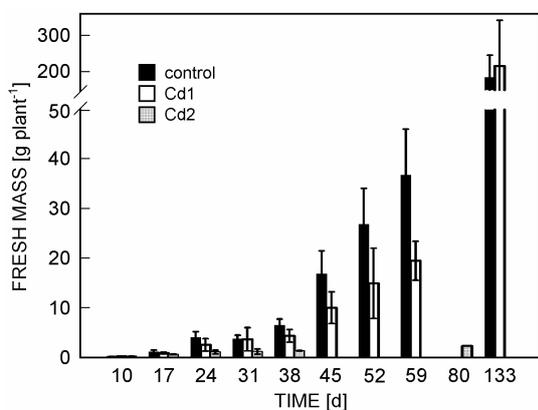


Fig. 1. Fresh mass accumulation (*n* = 3 - 6, *n* = 1 for Cd2 plant 80 d after sowing).

saturation of Cd in the stem over time was observed for the Cd2 plants, but not for Cd1 plants.

At the end of the experiment, an average amount of 832  $\mu\text{g}(\text{Cd})$  per plant were accumulated in the aerial parts of matured Cd1 plants. Extrapolating this (density 100 plants  $\text{m}^{-2}$ ), the phytoextraction potential was approximately 830  $\text{g}(\text{Cd}) \text{ha}^{-1}$  vegetation period<sup>-1</sup>.

The negative influence of Cd on chloroplasts was clearly visible through chlorosis of the leaves of the Cd2 plant (Fig. 3). After 20 d, the Chl content of the Cd2

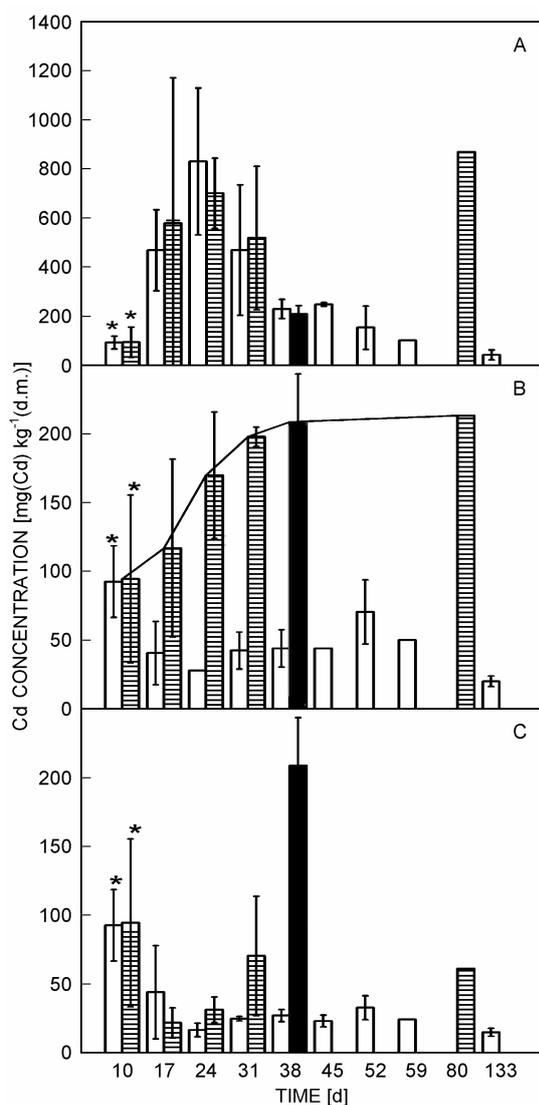


Fig. 2. Cd accumulation in roots (A), stems (B) and leaves (C) of hemp plants grown on cadmium contaminated soil. Cd determination 10 d after sowing was carried out with the whole plant, separation in different plant parts was not possible. The Cd concentration of the Cd2 plant 38 d after sowing was markedly different because it primarily represents the stem (open columns - Cd1, striped columns - Cd2, closed columns - primarily stem, \* - whole plants) ( $n = 2 - 6$ ,  $n = 1$  for Cd2 plant 80 d after sowing).

plants was lower than 30 % of that in the control plants. At the end of the trail, the leaves of Cd2 were mostly necrotic. For the first 10 weeks, the control plants had higher Chl contents than the Cd1 plants, and with the exception of day 20, this was not significant. At the end of the trail, the Cd1 values were higher in comparison to the control; however, values were not significant.

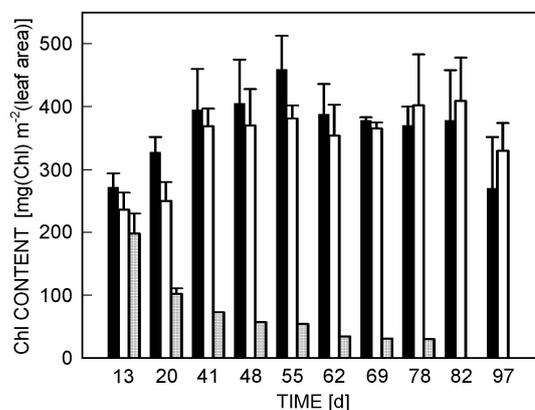


Fig. 3. Chlorophyll content during the 97-d trail (closed columns - control, open columns - Cd1, grey columns - Cd2) ( $n = 3 - 6$ ,  $n = 1$  for Cd2 plants after day 20).

Under greenhouse conditions, we observed that the Cd1 plants were not negatively influenced by the Cd treatment (Fig. 4). During the whole experiment,  $F_0$ ,  $F_v$  and  $F_v/F_m$  were in the same range as those of the control plants. In contrast, the Cd2 plant exhibited, with fluctuations, decreases of  $F_0$  and  $F_v$ ; however,  $F_v/F_m$  was only slightly reduced.

The control and the Cd1 plants always had very similar high values of  $\Phi_{PS2}$ ,  $q_p$  and  $F_v'/F_m'$ , in contrast to the Cd2 plants, for which values were mostly significantly lower or differed drastically (Fig. 5).

Light response experiments were used to determine the best suited PPFD for the examination of Cd effects on the photosynthesis apparatus (Fig. 6). At 500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD, there is a moderate, weak stress which produces electron pressure in the ET chain ( $q_p \approx 0.6$ , Öquist *et al.* 1992) coupled with the formation of a proton gradient ( $\Delta p\text{H}$ ) across the thylakoid membranes, as demonstrated by  $q_E$  and  $q_F$  formation, respectively. Since PPFD 500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  caused mild light stress and a  $q_F$  formation that led to  $F_v'/F_m'$  and  $q_F$  values near the breakpoint where regulation of PS 2 alters (Fig. 6, with arrow marked point), this PPFD was selected to analyse heavy metal influence on photosynthesis. Under these conditions, even small changes due to Cd would be expected to have a great impact on quenching parameters.

The data calculated for the control and the Cd1 plants fitted well with the biphasic curve of  $F_v'/F_m'$  vs.  $q_F$  dependence (Fig. 6) and verified the light response experiments. In contrast, the Cd2 values differed

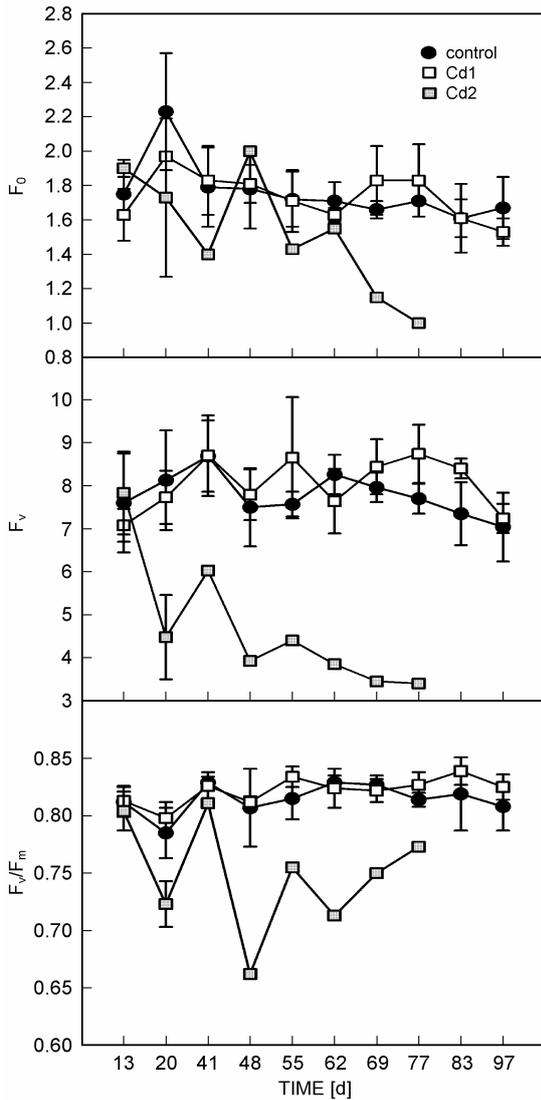


Fig. 4. Parameters of chlorophyll fluorescence ( $F_0$ ,  $F_v$ ,  $F_v/F_m$ ) during the 97-d trail ( $n = 3 - 6$ ,  $n = 1$  for Cd2 plants after day 20).

considerably, demonstrating the strong impact of Cd on hemp photosynthetic regulation.

Non-photochemical quenching,  $q_N$  (Fig. 7), and its components  $q_F$  and  $q_I$  showed a high degree of oscillations, with values of these parameters running in parallel for both the control and the Cd1 plants, without

**Discussion**

In hemp, we observed an inhibitory effect of Cd on plant growth (Fig. 1), but no negative effect on germination. The higher germination rates on Cd-contaminated soils are unexpected, but similar observation was also reported by Seregin and Ivanov (2001). Conceivably, the higher germination rates are due to the expression of stress

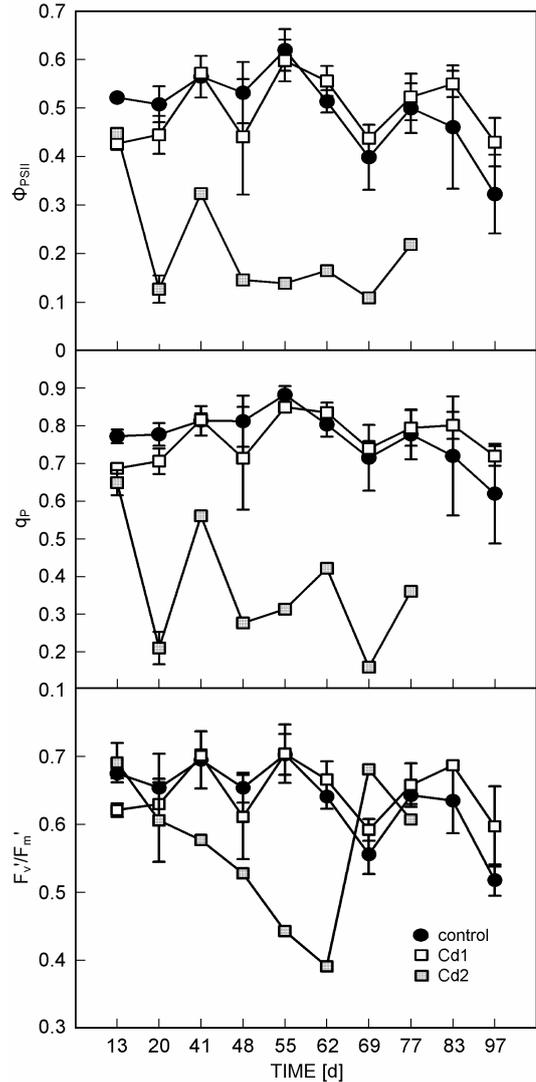


Fig. 5. Parameters of chlorophyll fluorescence ( $\Phi_{PSII}$ ,  $q_p$ ,  $F_v'/F_m'$ ) during the 97-d trail ( $n = 3 - 6$ ,  $n = 1$  for Cd2 plants after day 20).

any significant difference. However,  $q_N$ ,  $q_F$  and  $q_I$  of the Cd2 plants revealed somewhat of an inverse behaviour.  $q_I$  of the Cd2 plants displayed an enhanced susceptibility of the Cd2 plants to photoinhibition. Finally, the oscillations of all analysed parameters (Figs. 4,5,7) reflected changes in plant photosynthetic performance during the vegetation period.

inducible genes during germination; thereby, young seedlings are protected and the metabolism of young plants is better adjusted to the harsh conditions, allowing them to better survive the first days after germination than plants growing on non-contaminated soil.

Growth on high Cd concentrations leads to a

significant loss of vitality and biomass production (Fig. 1); however, hemp can tolerate Cd soil concentration of up to 17 mg(Cd) kg<sup>-1</sup>(soil) without any major effect on total biomass production (Cd1 plants). The origin of growth inhibition could not be conclusively determined, but the roots seem to be a critical place of Cd action as Cd concentrations in Cd1 and Cd2 plant roots were identical, although growth was only strongly inhibited in Cd2 plants (Fig. 2).

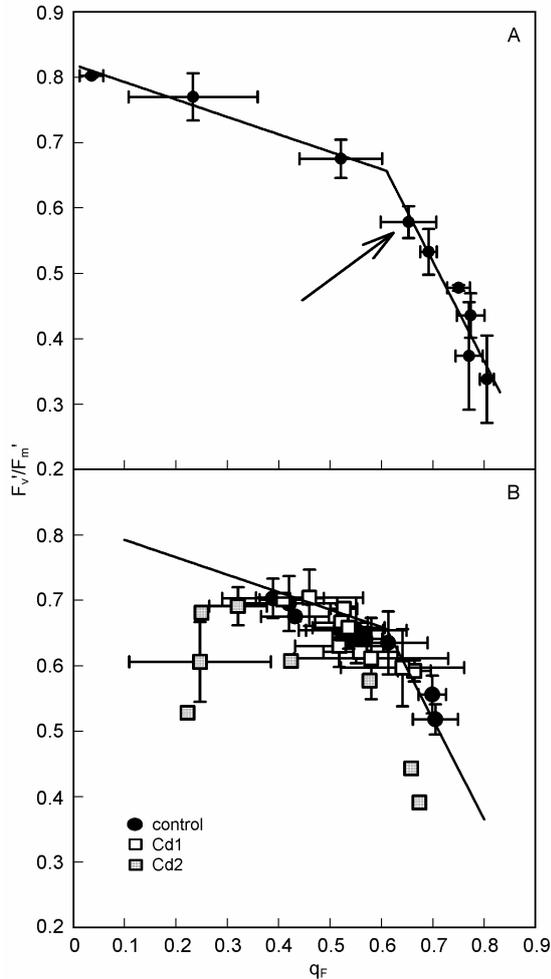


Fig. 6.  $F_v'/F_m'$  vs.  $q_F$  as summarised from the light response experiments (A) and cadmium trial (B). Arrow in A marks PPFD of 500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  which was used for the determinations of Cd effects. The regression line represents the PPFD response data.

Hemp roots demonstrated a strong resistance to heavy metals (Fig. 2) and have shown a somewhat hyperaccumulator-like potential (more than 100 mg kg<sup>-1</sup> Cd in dry tissue); however, this seemed to depend on the plant development stage. These high values of Cd accumulation cannot be explained exclusively by passive ion uptake. Immobilisation, by binding to the cell walls, might play a minor role (Sanita di Toppi and Gabbriellini 1999), but this does not account for hyperaccumulation. Juvenile roots accumulate Cd, and therefore, hemp may

use phytochelatins in a detoxification process by sequestering Cd and rendering it harmless by transporting and storing it in vacuoles (Zenk 1996). Salt *et al.* (1995b) reported strong Cd uptake in *Brassica juncea* and *Thlaspi caerulescens* growing in a poorly polluted nutrition solution, which was associated with rapid phytochelatin accumulation in the roots. The decrease of Cd concentrations in older roots is caused by a biological dilution effect due to root growth and is linked with the loss of hyperaccumulation ability.

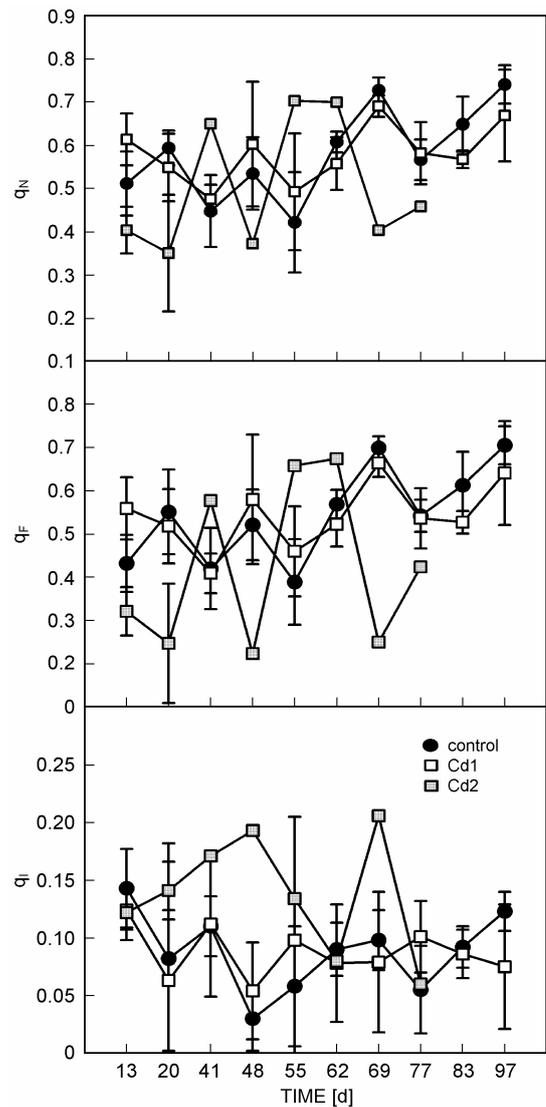


Fig. 7. Fluorescence quenching ( $q_N$ ,  $q_F$  and  $q_I$  values) during the 97-d trail ( $n = 3 - 6$ ,  $n = 1$  for Cd2 plants after day 20).

The high values of Cd in the roots reflect the genetically determined capability of hemp roots for accumulation and detoxification as maintained in the Cd1 plants. The fact that the Cd concentrations in roots of the Cd1 and Cd2 plants are equal, but not observed in stems and leaves, is based on the breakdown of the control mechanisms for Cd uptake in the endodermis, transport

and xylem loading. Under the Cd2 soil conditions, the system breaks down and uncontrolled Cd uptake in xylem and stem occurred. The soil pH seemed to be of importance for these control mechanisms because root Cd concentrations are equal but soil pH differs in Cd1 and Cd2 soil.  $H^+$ -ATPase,  $Cd^{2+}$  transporting P-Type ATPase and  $Ca^{2+}$ -channels might be involved in this control (Greger 1999, Axelsen and Palmgren 2001).

There are two conceivable reasons for growth impairment in the Cd2 plants. First, Cd damages the capability of cell division of meristematic cells which is highly plausible after the many reports on the adverse effects of heavy metals on cell division and elongation (Dietz *et al.* 1999, Rama Devi and Prasad 1999, Jiang and Liu 2000, Liu *et al.* 2003/4).

However, this seems to be only applicable to leaves and stems, but not for roots, because root growth of Cd1 plants was not inhibited. Hence, if the meristems are the main destination for Cd, then there must be a strong difference between the meristems of the stem, leaves and roots. Second, the high heavy metal concentrations in stems and leaves of the Cd2 plants lead to inhibition of photosynthesis, as has been described previously (Krupa and Baszynski 1995, Dubey 1997, Dietz *et al.* 1999, Prasad and Strzalka 1999, Siedlecka and Krupa 1999).

$F_0$  and  $F_v$  provided an insight into the Cd effect on PS 2. No difference between control and the Cd1 plants was observed; in stark contrast, the Cd2 plants showed a decline of  $F_0$  and  $F_v$  (Fig. 4). A decrease of  $F_v$  indicates an inhibition of the water splitting complex (Weis and Berry 1988, Ouzounidou *et al.* 1997). Cd acts on the water splitting complex of the Cd2 plants, and as a consequence, electron donation to PS 2 is inhibited. A  $F_0$  decrease, as also observed here, might result from lower fluorescence yield because of reduced chlorophyll molecules in PS 2 antenna, which is in accordance with Ouzounidou *et al.* (1997), who described this phenomenon for wheat growing in a Cd-containing solution. The impairment of Chl synthesis in Cd2 plants is evidenced by a marked chlorosis (Fig. 3). A direct effect on Chl synthesis, light harvesting complex size and/or chloroplast density seems to be the reason and is also responsible for  $F_0$  decrease. Under greenhouse conditions, chlorophyll destruction due to oxidative stress was ruled out, because of only weak photoinhibition (Fig. 4,  $F_v/F_m$ ). The coefficient  $F_v/F_m$  is known to be a more insensitive indicator for stress, but harsh conditions leading to oxidative stress will lead to low  $F_v/F_m$  values. Strong photoinhibition events are accompanied by  $F_v/F_m$  values  $< 0.5$  (Hetherington *et al.* 1989, Greer *et al.* 1991, Öquist *et al.* 1993). Here, the Cd2 plants obtained  $F_v/F_m$  values in the range of 0.66 to 0.81, which indicate no strong photoinhibition.

In addition, the Cd2 plants showed significantly lower  $\Phi_{PS2}$ ,  $q_p$  and  $F_v'/F_m'$  values (Fig. 5) than control or Cd1 plants, which infer the negative impact of Cd on photosynthesis. The finding that  $F_v'/F_m'$  increases at days

69 and 77 cannot be explained. The loss of photosynthesis performance  $\Phi_{PS2}$  (Fig. 5) was not only caused by a decrease of  $F_v'/F_m'$  (most of the time), but also of  $q_p$ , which indicates a reduced consumption of NADPH and ATP in the Calvin cycle due to a reduced demand of sugars in other plant parts caused by Cd or an inhibition of enzymes of the Calvin cycle (Weigel 1985, Stiborova 1988, Sheoran *et al.* 1990, Chugh and Sawhney 1999). The impairment of photosynthesis and chlorophyll synthesis with a subsequent lack of sugar production, and possibly, an inhibited of cell division are the reasons for growth stagnation and mortality of Cd2 plants.

The Cd1 plants demonstrate an overall strong tolerance towards Cd. Only in the early phase of the trial a minor inhibition of growth (Fig. 1) and chlorophyll synthesis (Fig. 3) was detected. Cd uptake in leaves had no significant influence on Chl synthesis in the Cd1 plants, except on day 20 after sowing (Fig. 3). However, there seemed to be a moderate reduction in the early growth stages. This could be either because of decreased synthesis through inhibition or as a consequence of oxidative damage of Chl. However, for reasons as previously stated here, oxidative damage can again ruled out (see above). Competition between Cd and nutrients (Yang *et al.* 1996, Ouzounidou *et al.* 1997, Siedlecka and Krupa 1999) or inhibition of enzymes for chlorophyll synthesis (Stobart *et al.* 1985, Böddi *et al.* 1995) are the reasons for reduced capability of Chl synthesis in the early stage of Cd1 growth. The control and the Cd1 plants displayed the same photosynthetic fitness, and no significant difference could be detected for  $\Phi_{PS2}$  and  $q_p$  (Fig. 5). Therefore, the growth depression in early growth stages of the Cd1 plants does not originate from photosynthesis disturbance. Enhanced photorespiration and respiration might be candidates for the loss of fixed  $CO_2$ , resulting in growth inhibition. However, most of the available data do not support this as Löscher and Köhl (1999), Seregin and Ivanov (2001) and Bansal *et al.* (2002) all reported Cd to inhibit both respiration and photorespiration. In support, however, Lee *et al.* (1976) documented an increase in respiration rate in soybean treated with Cd, and Lunackova *et al.* (2003) found a significant increase of root respiration for different *Salix* species induced by Cd. A limited transport of sugars from leaves to shoots or roots as well as limited plant needs for sugars might be of minor importance. An accumulation of sugars in the leaves and chloroplasts, possibly accompanied with phosphate depletion, would generate a considerably down-regulation of photosynthesis, which would become apparent *via* a significant decline of  $\Phi_{PS2}$  and  $F_v'/F_m'$  (Fig. 5) and an increase of  $q_N$  and  $q_F$  (Fig. 7). The growth depression in the early stage of the trial is due to an enhanced demand for sugars in roots and for ATP synthesis *via* respiration used for detoxification (Lunackova *et al.* 2003) and acclimation. In the later phase of the vegetation period (after day 60), a long-term acclimation of the Cd1 plants became evident. This

acclimation is demonstrated by an increase compared to the control, of biomass formation (Fig. 1), Chl content (Fig. 3) and also of  $\Phi_{PS2}$ ,  $q_P$  and  $F_v'/F_m'$  (Fig. 5). Taken together, these observations indicate enhanced photosynthetic performance of the Cd1 plants. However, the increases of these parameters are not significant; the sum of these increases can be taken as an indication of long-term acclimation.

The efficiency of open PS 2 reaction centres, as demonstrated here, is under the control of mechanisms involved in  $q_F$  formation (Fig. 6). Under conditions of high photosynthetic performance, *i.e.* high  $F_v'/F_m'$  and low  $q_F$  values, indicating low energisation as well as a small  $\Delta pH$ , regulation of PS 2 efficiency is poor, and could be termed "low energy regulation". This "low energy regulation" allows optimal use of light *via* electron transport chain to produce maximum amounts of NADPH and ATP. However, a sudden threshold exists in the regulation of PS 2, *i.e.* for  $q_F > 0.6$ ,  $F_v'/F_m'$  decreases drastically with further increase of  $q_F$ , which is associated with a strong energisation of photosynthesis apparatus; this state could be described as "high energy regulation" of PS 2. The "high energy regulation" is required because of the onset of light stress when the ET chain becomes increasingly reduced, *i.e.*  $q_P < 0.6$  (Öquist *et al.* 1992). Downregulation of PS 2 efficiency is necessary to reduce electron pressure in electron transport chain and switch over energy consumption to heat dissipation.

The regulation of the efficiency of PS 2 via "low" and "high energy regulation" must be mediated by two or more mechanisms, where zeaxanthin formation and conformational changes of PS 2 might be involved (Horton *et al.* 1996). The threshold between "low" and "high" energy regulation may reflect 1) a critical  $\Delta pH$  for conformational changes, 2) the onset of conformational changes, or 3) the start of zeaxanthin synthesis via the xanthophyll cycle.

The control and the Cd1 plants had the same regulation pattern as observed in light response experiments (Fig. 6), demonstrating the regulatory features of photosynthesis of hemp. In contrast, the Cd2 plants differed strongly, with high Cd concentrations in leaves resulting in a complex change of photosynthetic regulation in hemp. It became apparent that Cd not only disturbs the water splitting complex (see above), but also mechanisms that are involved in  $q_N$  and  $q_F$  formation and heat dissipation (Fig. 6).

The  $q_N$ ,  $q_F$  and  $q_I$  formation themselves are not inhibited by Cd. Although all the parameters were distinct, in the Cd2 plants these parameters were counter to those determined for the control and the Cd1 plants

(Fig. 7). Moreover, since electron transport and  $\Delta pH$  build-up take place in the Cd2 plants as well as in the control and the Cd1 plants, it is unlikely that the thylakoid membranes and their integrity are the main targets of deleterious Cd action in hemp chloroplasts.

The changes in the regulation of PS 2 in the Cd2 plants (Fig. 6) could be caused by structural alterations of PS 2 reactions centres and/or antenna complex resulting in changes in the induction of energy dissipation, or a disturbance of phosphorylation of the light-harvesting complex with subsequent changes in state 1-state 2 transition. However, it is also likely that the effects on the violaxanthin cycle cause changes in the Cd2 plants. Energy dissipation originates in antenna as well as in the reaction centres (Heber *et al.* 2001), and possibly Cd blocks one compound of energy dissipation more strongly than another. Zeaxanthin is an important, but not indispensable factor for energy dissipation (Schreiber and Neubauer 1990). Therefore, Cd could interfere with the violaxanthin cycle and inhibit zeaxanthin synthesis, causing changes in the quenching properties. Barylta *et al.* (2001) observed a reduction of violaxanthin cycle pigments of nearly 50 % in rape leaves, supporting an unfortunate effect of Cd on zeaxanthin synthesis. In addition, tomato and soybeans plants also undergo a reduction of carotenoid contents when treated with Cd (Baszynski *et al.* 1980, El-Shintinawy 1999).

It remains to be clarified how high Cd concentrations affect the energy dissipation of PS 2; however, a worse synergetic effect associated with structural changes of PS 2 and impairment of the violaxanthin cycle is credible.

Nevertheless, hemp has been shown to be a suitable candidate for phytoremediation approaches. Cv. USO31 is able to extract, on average, up to 830 g(Cd) ha<sup>-1</sup> vegetation period<sup>-1</sup>. This is approximately 6.5 times higher than that of hemp growing under natural conditions with 126 g(Cd) ha<sup>-1</sup> vegetation period<sup>-1</sup> (Linger *et al.* 2002). For *Thlaspi caerulescens* under optimal growth conditions, up to 2 kg(Cd) ha<sup>-1</sup> yr<sup>-1</sup> were extracted (Saxena *et al.* 1999). Furthermore, Robinson *et al.* (1998) extrapolated the extraction potential of *T. caerulescens* from a pot trial to be 8.4 kg(Cd) ha<sup>-1</sup> yr<sup>-1</sup> under fertilised conditions. These figures are 2.4 and 10 times higher than USO31 (Cd1), respectively. However, hemp is much better suited for phytoextraction than most other crops and wild plants with calculated extraction potentials of between 2 and 222 g(Cd) ha<sup>-1</sup> yr<sup>-1</sup> (Felix 1997). The advantage in using hemp as opposed to *T. caerulescens* is the higher biomass yield, which could be used for energy generation in power stations and combines ecological aspects with economic requirements.

## Conclusion

Hemp is a Cd-tolerant plant, with strong resistant roots and the capability for long-term acclimation. These

characteristics endorse hemp as a key candidate for phytoextraction approaches. For plant survival, the control

of cadmium transport to stems and leaves is highly critical. When Cd concentrations in leaves exceed a threshold, PS 2 is influenced in a complex manner,

chlorophyll synthesis, water splitting, Calvin cycle enzymes and regulation of energy distribution of PS 2 are effected.

## References

- Adriano, D.C.: Trace Elements in the Terrestrial Environment. - Springer, Berlin 1986.
- Allaway, W.H.: The environmental cycling of trace elements. - Adv. Agron. **20**: 235-274, 1968.
- Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.
- Axelsen, B.A., Palmgren, M.G.: Inventory of the superfamily of P-type ion pumps in *Arabidopsis*. - Plant Physiol. **126**: 696-706, 2001.
- Bansal, P., Sharma, P., Goyal, V.: Impact of lead and cadmium on enzyme of citric acid cycle in germinating pea seeds. - Biol. Plant. **45**: 125-127, 2002.
- Baryla, A., Carrier, P., Franck, F., Coulomb, C., Sahut, C., Havaux, M.: Leaf chlorosis in oilseed rape plants (*Brassica napus*) grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. - Planta **212**: 696-709, 2001.
- Baszynski, T., Wajda, L., Krol, M., Wolinska, D., Krupa, Z., Tukendorf, A.: Photosynthetic activities of cadmium-treated tomato plants. - Physiol. Plant. **48**: 365-370, 1980.
- Björkman, O., Demmig, B.: Photon yield of O<sub>2</sub> evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants diverse origins. - Planta **170**: 489-504, 1987.
- Böddi, B., Oravecz, A.R., Lehoczki, E.: Effect of cadmium on organization and photoreduction of protochlorophyllide in dark-grown leaves and etioplast inner membrane preparations of wheat. - Photosynthetica **31**: 411-420, 1995.
- Bolhär-Nordenkamp, H.R., Long, S.P., Lechner, E.G.: Die Bestimmung der Photosynthesekapazität über die Chlorophyllfluoreszenz als Maß für die Streßbelastung von Bäumen. - Phytol. **29**: 119-135, 1989.
- Chugh, L.K., Sawhney, S.K.: Photosynthetic activities of *Pisum sativum* seedlings grown in presence of cadmium. - Plant Physiol. Biochem. **37**: 297-303, 1999.
- Chronopoulos, J., Haidouti, C., Chronopoulou-Sereli, A., Massas, I.: Variations in plant and soil lead and cadmium content in urban parks in Athens, Greece. - Sci. total Environ. **196**: 91-98, 1997.
- Dahmani-Muller, H., Van Oort, F., Balabane, M.: Strategies of heavy metal uptake by three plant species growing near a metal smelter. - Environ. Pollut. **109**: 231-238, 2000.
- Dietz, K.-J., Baier, M., Krämer, U.: Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. - In: Prasad, M.N.V., Hagemeyer, J. (ed.): Heavy Metal Stress in Plants. Pp. 73-98. Springer, Heidelberg 1999.
- Dubey, R.S.: Photosynthesis in plants under stressful conditions. - In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 859-876. Marcel Dekker, New York 1997.
- El-Shintinawy, F.: Glutathione counteracts the inhibitory effect induced by cadmium on photosynthetic process in soybean. - Photosynthetica **36**: 171-179, 1999.
- Ernst, W.: Physiological and biochemical aspects of metal tolerance. - In: Mansfield, T.A. (ed.): Effects of Air Pollutants on Plants. Pp. 115-133. Cambridge University Press, Cambridge 1976.
- Ernst, W.H.O.: Bioavailability of heavy metals and decontamination of soils by plants. - Appl. Geochem. **11**: 163-167, 1996.
- Felix, H.: Field trials for *in situ* decontamination of heavy metal polluted soils using crops of metal-accumulating plants. - Z. Pflanzenernähr. Bodenk. **160**: 525-529, 1997.
- Foy, C.D., Chaney, R.L., White, M.C.: The physiology of metal toxicity in plants. - Ann. Rev. Plant Physiol. **29**: 511-566, 1978.
- Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - Biochim. biophys. Acta **990**: 87-92, 1989.
- Greer, D.H., Ottander, C., Öquist, G.: Photoinhibition and recovery of photosynthesis in intact barley leaves at 5 °C and 20 °C. - Physiol. Plant. **81**: 203-210, 1991.
- Greger, M.: Metal availability and bioconcentration in plants. - In: Prasad, M.N.V., Hagemeyer, J. (ed.): Heavy Metal Stress in Plants. Pp. 1-28. Springer, Heidelberg 1999.
- Heber, U., Bukhov, N.G., Shuvalov, V.A., Kobayashi, Y., Lange, O.L.: Protection of the photosynthetic apparatus against damage by excessive illumination in homoiohydric leaves and poikilohydric mosses and lichens. - J. exp. Bot. **52**: 1999-2006, 2001.
- Hetherington, S.E., He, J., Smillie, R.M.: Photoinhibition at low temperature in chilling-sensitive and -resistant plants. - Plant Physiol. **90**: 1609-1615, 1989.
- Horton, P., Ruban, A.V., Walters, R.G.: Regulation of light harvesting in green plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **47**: 655-694, 1996.
- Horvath, G., Droppa, M., Oravecz, A., Raskin, V.I., Marder, J.B.: Formation of the photosynthetic apparatus during greening of cadmium-poisoned barley leaves. - Planta **199**: 238-244, 1996.
- Jiang, W., Liu, D.: Effects of Pb<sup>2+</sup> on root growth, cell division, and nucleolus of *Zea mays* L. - Bull. Environ. Contam. Toxicol. **65**: 786-793, 2000.
- Kevrešan, S., Kiršek, S., Kandrač, J., Petrović, N., Kelemen, Dj.: Dynamics of cadmium distribution in the intercellular space and inside cell in soybean roots, stems and leaves. - Biol. Plant. **46**: 85-88, 2003.
- Khudsar, T., Mahmooduzzafar, Iqbal, M., Sairam, R.K.: Zinc-induced changes in morpho-physiological and biochemical parameters in *Artemisia annua*. - Biol. Plant. **48**: 255-260, 2004.
- Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. - Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.
- Krupa, Z., Baszynski, T.: Some aspects of heavy metal toxicity towards photosynthetic apparatus - direct and indirect

- effects on light and dark reaction. - *Acta Physiol. Plant.* **17**: 177-190, 1995.
- Lee, K.C., Cunningham, B.A., Paulsen, G.M., Liang, G.H., Moore, R.B.: Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. - *Physiol. Plant.* **36**: 4-6, 1976.
- Linger, P., Brüggemann, W.: Correlations between chlorophyll fluorescence quenching parameters and photosynthesis in a segregating *Lycopersicon esculentum* × *L. peruvianum* population as measured under constant conditions. - *Photosynth. Res.* **61**: 145-156, 1999.
- Linger, P., Müssig, J., Fischer, H., Kobert, J.: Industrial hemp (*Cannabis sativa* L.) growing on heavy metal contaminated soil: fibre quality and phytoremediation potential. - *Ind. Crops Prod.* **16**: 33-42, 2002.
- Liu, D., Jiang, W., Gao, X.: Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. - *Biol. Plant.* **47**: 79-83, 2003/4.
- Lösch, R., Köhl, K.I.: Plant respiration under the influence of heavy metals. - In: Prasad, M.N.V., Hagemeyer, J. (ed.): *Heavy Metal Stress in Plants*. Pp. 139-156. Springer, Heidelberg 1999.
- Lunackova, L., Masarovicova, E., Kral'ova, K., Stresko, V.: Responses of fast growing woody plants from family Salicaceae to cadmium treatment. - *Bull. Environ. Contam. Toxicol.* **70**: 576-585, 2003.
- Mazen, A.M.A.: Accumulation of four metals in tissues of *Corchorus olitorius* and possible mechanisms of their tolerance. - *Biol. Plant.* **48**: 267-272, 2004.
- Öquist, G., Chow, W.S., Anderson, J.M.: Photoinhibition of photosynthesis represents a mechanism for long-term regulation of photosystem II. - *Planta* **186**: 450-460, 1992.
- Öquist, G., Hurry, V.M., Huner, N.P.A.: The temperature dependence of the redox state of  $Q_A$  and susceptibility of photosynthesis to photoinhibition. - *Plant Physiol. Biochem.* **31**: 683-691, 1993.
- Ouzounidou, G., Moustakas, M., Eleftheriou, E.P.: Physiological and ultrastructural effects of cadmium on wheat (*Triticum aestivum* L.) leaves. - *Arch. Environ. Contam. Toxicol.* **32**: 154-160, 1997.
- Prasad, M.N.V.: Cadmium toxicity and tolerance in vascular plants. - *Environ. exp. Bot.* **35**: 525-545, 1995.
- Prasad, M.N.V., Strzalka, K.: Impact of heavy metals on photosynthesis. - In: Prasad, M.N.V., Hagemeyer, J. (ed.): *Heavy Metal Stress in Plants*. Pp. 117-138. Springer, Heidelberg 1999.
- Rama Devi, S., Prasad, M.N.V.: Membrane lipid alterations in heavy metal exposed plants. - In: Prasad, M.N.V., Hagemeyer, J. (ed.): *Heavy Metal Stress in Plants*. Pp. 99-116, Springer, Heidelberg 1999.
- Robinson, B.H., Leblanc, M., Petit, D., Kirkham, K.H., Gregg, P.E.H.: The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. - *Plant Soil* **203**: 47-56, 1998.
- Ruban, A.V., Horton, P.: Regulation of non-photochemical quenching of chlorophyll fluorescence in plants. - *Aust. J. Plant Physiol.* **22**: 221-230, 1995.
- Salt, D.E., Blaylock, M., Kumar, N.P.B.A., Dushenkov, V., Ensley, B.D., Chet, I., Raskin, I.: Phytoremediation: a novel strategy for removal of toxic metals from environment using plants. - *Biotechnology* **13**, 468-474, 1995a.
- Salt, D.E., Prince, R.C., Pickering, I.J., Raskin, I.: Mechanisms of cadmium mobility and accumulation in Indian mustard. - *Plant Physiol.* **109**: 1427-1433, 1995b.
- Sanita di Toppi, L., Gabbriellini, R.: Response to cadmium in higher plants. - *Environ. exp. Bot.* **41**: 105-130, 1999.
- Saxena, P.K., Krishna Raj, S., Perras, M.R., Vettakkorumakankav, N.N.: Phytoremediation of heavy metal contaminated and polluted soils. - In: Prasad, M.N.V., Hagemeyer, J. (ed.): *Heavy Metal Stress in Plants*. Pp. 305-329. Springer, Heidelberg 1999.
- Schreiber, U., Bilger, W.: Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. - In: Tenhunen, J.D., Catarino, F.M., Lange, O.L., Oechel, W.D. (ed.): *Plant Response to Stress*. Pp. 27-53. Springer, Heidelberg 1987.
- Schreiber, U., Neubauer, C.:  $O_2$ -dependent electron flow, membrane energization and the mechanisms of nonphotochemical quenching of chlorophyll fluorescence. - *Photosynth. Res.* **25**: 279-293, 1990.
- Schreiber, U., Schliwa, U., Bilger, W.: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. - *Photosynth. Res.* **10**: 51-62, 1986.
- Seregin, I.V., Ivanov, V.B.: Physiological aspects of cadmium and lead toxic effects on higher plants. - *Russ. J. Plant Physiol.* **48**: 523-544, 2001.
- Sheoran, I.S., Singal, H.R., Singh, R.: Effect of cadmium and nickel on photosynthesis and the enzymes of photosynthetic carbon reduction cycle in pigeon-pea (*Cajanus cajan* L.). - *Photosynth. Res.* **23**: 345-351, 1990.
- Siedlecka, A., Krupa, Z.: Cd/Fe interaction in higher plants - its consequences for the photosynthetic apparatus. - *Photosynthetica* **36**: 321-331, 1999.
- Šimonovičová, M., Tamás, L., Huttová, J., Mistrík, I.: Effect of aluminium on oxidative stress related enzymes activities in barley roots. - *Biol. Plant.* **48**: 261-266, 2004.
- Stiborova, M.:  $Cd^{2+}$  ions affect the quaternary structure of ribulose-1,5-bisphosphate carboxylase from barley leaves. - *Biochem. Physiol. Pflanz.* **183**: 371-378, 1988.
- Stobart, A.K., Griffiths, W.T., Ameen-Bukhari, I., Sherwood, R.P.: The effect of  $Cd^{2+}$  on biosynthesis of chlorophyll in leaves of barley. - *Physiol. Plant.* **63**: 293-298, 1985.
- Walters, R.G., Horton, P.: Resolution of components of non-photochemical chlorophyll fluorescence quenching in barley leaves. - *Photosynth. Res.* **27**: 121-133, 1991.
- Weigel, H.J.: The effect of  $Cd^{2+}$  on photosynthetic reactions of mesophyll protoplasts. - *Physiol. Plant.* **63**: 192-200, 1985.
- Weis, E., Berry, J.A.: Plants and high temperature stress. - In: Longe, S.F., Woodward, F.I. (ed.): *Plants and Temperature*. Pp. 329-346. Company of Biologists Ltd, Cambridge 1988.
- Yang, X., Baligar, V.C., Martens, D.C., Clark, R.B.: Cadmium effects on influx and transport of mineral nutrients in plant species. - *J. Plant Nutr.* **19**: 643-656, 1996.
- Zenk, M.H.: Heavy metal detoxification in higher plants - a review. - *Gene* **179**: 21-30, 1996.